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REVIEW
of the doctoral dissertation of Pascaline Aimee Uwineza, M.Sc.

entitled „Use of natural extracts from *Lamium album* in the biological protection of cereals against *Fusarium* pathogens"

conducted in the Department of Chemistry
Faculty of Food Science and Nutrition Poznan Iniversity of Life Sciences
under the supervision of prof. dr hab. Agnieszka Waśkiewicz

The review was conducted on the basis of Resolution No. NZDT-4000-30/2024 of the Scientific Council of the Discipline of Food Technology and Nutrition of the University of Life Sciences in Poznan of 28 November 2024 on the appointment of reviewers of the doctoral thesis of Pascaline Aimee Uwineza, M.Sc.

The review of the dissertation submitted for evaluation has been prepared in accordance with the applicable legal regulations in this regard - the Act of 20 July 2018 Law on Higher Education and Science (Journal of Laws of 2020, item 85, as amended) and consists, as recommended by the Council for Scientific Excellence, of an assessment of the following elements:

1) whether the dissertation demonstrates the applicant's general theoretical knowledge in the discipline of food technology and nutrition;
2) whether the doctoral dissertation demonstrates the applicant's ability to conduct the research work independently;
3) whether the dissertation demonstrate an original solution to a scientific problem, an original solution to the application of the results of one's own research in the economic or social sphere;
4) final evaluation.

Whether the dissertation demonstrates the applicant's general theoretical knowledge in the discipline of food technology and nutrition?

In recent years, there has been a growing interest in the use of natural plant extracts to protect cereals against *Fusarium* pathogens. This interest can be attributed to concerns about the negative impact of synthetic fungicides on human health and the environment. Pesticides play a pivotal role in protecting crops from pests, diseases and weeds; however, their utilisation is also associated with numerous detrimental effects on the environment, human health and beneficial organisms. The

overuse or misapplication of these products can lead to grave consequences, underscoring the necessity to curtail their use and adopt more sustainable agricultural practices.

Agriculture is pivotal in terms of its role as the primary source of food resources for global populations. It is a fundamental sector of the economy, responsible for the production of food, industrial raw materials and animal feed. Its development and efficiency are essential to meet the growing demand for food in the context of global population growth. Cereals are the foundation of the global food system and are a key element in the fight against hunger and in ensuring food security for a growing population. According to the Food and Agriculture Organization of the United Nations (FAO), cereals are responsible for approximately 50% of the world's total caloric intake. Given their pervasive utilization and economic significance, the advancement of cereal production is imperative to satisfy future food requirements. Nevertheless, these pivotal crops are confronted with substantial challenges posed by phytopathogenic fungi, which contribute considerably to global food losses exacerbated by the effects of climate change and prevailing environmental concerns.

Mycotoxins are toxic metabolic by-products of certain molds that have the potential to induce pathological changes in humans, animals and plants. To date, several hundred toxic substances produced by moulds belonging to the species *Aspergillus*, *Penicillium* and *Fusarium* have been identified and are of particular relevance in research. Mycotoxin contamination of food represents a serious problem for agriculture and the food industry worldwide. The most significant risk to humans arises from the ingestion of contaminated foodstuffs. Mycotoxins have been detected in a wide range of plant products, including cereals, oilseeds, coffee, fruits, spices and nuts, as well as in animal products such as milk, meat and offal. In recent years, advancements in food toxicology and modern analytical methods have enabled greater focus on the toxins produced by the genus *Fusarium*, which are commonly found in temperate climates, including Europe. These mycotoxins represent a diverse group of compounds with a broad spectrum of toxic properties. The growth of *Fusarium* fungi and the production of mycotoxins are contingent on a number of environmental factors, including the composition and consistency of the substrate, the presence of micronutrients and associated microflora. Humidity and temperature are also crucial factors, directly affecting the intensity of growth and toxigenicity.

As the PhD student correctly observed, given that wheat is one of the most significant cereals globally in terms of both cultivated area and nutritional importance, and is a staple in the diet of billions of people, as well as a raw material in numerous food industries, its protection against *Fusarium* pathogens and their toxic metabolites is imperative for global food security. An alternative to synthetic fungicides has been proven to be the use of extracts from various plant parts, including roots, bark, seeds, shoots, leaves, fruits, flowers, cloves, rhizomes or stems. The advantage of using such a mixture of compounds, including phenolic acids, flavonoids, tannins, terpenes and alkaloids, is that they can act as a natural fungicide. These compounds can act individually or in combination, thereby providing a synergistic effect that enhances the inhibition of the growth of phytopathogenic fungi during cultivation and after harvest.

A plethora of studies have recently underscored the antifungal potential of diverse plant extracts; however, the majority of these have been conducted *in vitro* and frequently neglect the impact on mycotoxin biosynthesis. Moreover, the extant literature signifies that the number of tested biopesticides based on plant extracts remains limited, whilst the demand for organic products continues to rise. One plant with as yet unexplored antimicrobial potential and a plethora of health benefits is *Lamium album*, commonly known as white buttercup or white nettle. As the author rightly points out, although a large number of studies have been conducted on its pharmacological properties,

little attention has been paid to the study of flower extracts as potential natural antifungal agents in agriculture. Furthermore, there are no studies in the available literature on the efficacy of *L. album* flower extracts against *Fusarium* pathogens *in vivo*. The extraction method has been identified as a critical factor influencing the efficiency of the process, and conventional techniques have been shown to be both time- and cost-intensive, resulting in the loss of volatile compounds, the degradation of thermally unstable compounds, and the lack of selectivity. To address these limitations associated with conventional methods, the PhD student employed supercritical fluid extraction (SFE).The research topics undertaken by the PhD student are of particular research and application interest and provide a valuable source of data to advance knowledge in this area. The author has identified a significant research area and has meticulously contributed to its development through her research, demonstrating a strong understanding of the subject matter and a high level of preparation. She has also employed a range of analytical methods to characterize the extracts. The research undertaken by the author has addressed a significant knowledge gap and, for the first time in the literature, has contributed to the development of research into biological alternatives to synthetic fungicides. The study emphasizes the importance of conducting comprehensive research prior to practical application in the agricultural environment.

Summary: the investigation undertaken in the dissertation of Pascaline Aimee Uwineza, M.Sc., into the efficacy of natural plant extracts derived from native herbaceous plants (*Lamium album*) in the biological protection of cereals against the agriculturally significant *Fusarium* pathogens *F. culmorum* and *proliferatum* is of considerable importance, pertinence and timeliness. This investigation is justified for cognitive and application reasons. The dissertation under review aligns with the prevailing themes in contemporary research within the discipline of food technology and nutrition.

Whether the doctoral dissertation demonstrates the applicant's ability to conduct the research work independently?

The revised dissertation of Pascaline Aimee Uwineza, M.Sc., entitled "Use of natural extracts from *Lamium album* in the biological protection of cereals against *Fusarium* pathogens" is a series of five thematically coherent original scientific papers carried out within the framework of the project funded by the National Science Centre in the OPUS 16 competition No. UMO-2018/31/B/NZ9/03485, published between 2020 and 2024 in journals indexed in Journal Citation Reports (Molecules, Applied Science, Toxins, Frontiers in Microbiology and Agriculture). The series has a total Impact Factor of 17.9 and a total number of Ministry of Science and Higher Education points (according to the year of publication) of 540. In all publications, the PhD student is the first and corresponding author, and her contribution is between 60 and 80%. It should also be mentioned that despite the fact that the works have been published relatively recently, they have already gained recognition, as evidenced by the number of citations in Scopus and Web of Science databases. The attached declarations of co-authors of the publication confirm the significant contribution of the author in the development of the study concept, methodology, performance of experiments and analyses, elaboration of study results, preparation of manuscripts, their improvement and editing, and validation of the analytical methods used.

The series of publications is accompanied by a 46-page summary in English, consisting of a theoretical introduction, research goals and hypotheses, methods, the most important results with the discussion, conclusions and references. These are preceded by a list of publications forming the basis of the dissertation, a list of abbreviations and an abstract (in English and Polish). Statements

of co-authors of publications and publications included in the dissertation are an integral part of the work.

The title of the dissertation is correctly worded and corresponds to the research results presented in the paper. The introduction aims to familiarize the reader with the issues concerning the use of natural plant extracts to protect cereals against *Fusarium* pathogens. The PhD student has set four research hypotheses and proposed five specific objectives, which are formulated correctly and logically as a result of the study of the current literature on the topic. The chapter entitled "Methods" contains synthesized information on the research material and methods used, presented in paragraphs. These issues are characterized in detail in the publications that form the subject of the dissertation. In the chapter "The most important results with the discussions", the PhD student presented a concise description of the results of the conducted research with a critical and scientific discussion. The statistical analysis of the results was conducted using conventional tests. The dissertation as a whole was summarized with five conclusions, and, as is worthy of note, it was stated which specific hypotheses were verified, a rarity in such works. The literature included in the dissertation comprises 108 English-language items presenting issues related to the topic of the study, of which almost 65% were published in the last decade.

The work has been written in accessible language, thereby enabling the results presented in the publications to be comprehended and analyzed.

Summary: I conclude that the dissertation submitted for review by Pascaline Aimee Uwineza, M.Sc., demonstrates her ability to conduct her research work independently and that it meets the formal requirements for dissertations for the doctoral degree.

Whether the dissertation demonstrate an original solution to a scientific problem, an original solution to the application of the results of one's own research in the economic or social sphere?

The objective of the research conducted by the PhD student was to evaluate the effectiveness of natural extracts from native herbaceous plants, particularly *Lamium album* flowers, in the biological protection of cereals against Fusarium pathogens. For this purpose, the author conducted multidirectional research, starting with the development of extraction conditions using a modern technique based on the use of supercritical carbon dioxide (SC-CO_2) and selecting, based on the results obtained (the effect of extraction parameters on the extracts composition and their antioxidant activity, extraction efficiency, composition and concentration of bioactive compounds), from among 4 families (*Lamiaceae*, *Rosaceae*, *Ranunculaceae* and *Elagnaceae*) one plant with promising antifungal potential - *Lamium album*. The antifungal properties of *L. album* flower extracts were then evaluated against *Fusarium* pathogens both *in vitro* (PDA medium) and *in vivo* (wheat pot and plot cultivation) experiments.

As outlined in the first publication (aut. Uwineza, P.A.; and Waśkiewicz, A. 2020. Recent Advances in Supercritical Fluid Extraction of Natural Bioactive Compounds from Natural Plant Materials. *Molecules* 25, 3847), a comprehensive review of the existing literature has been conducted, collating information on a technologically advanced approach to the extraction of natural bioactive components from plant-derived materials that is more environmentally friendly. Bioactive compounds of plant origin, which are present in minute quantities, have attracted considerable interest due to their beneficial effects on human health and their alignment with the prevailing trend of environmental sustainability. Various new extraction methods and conventional extraction methods have been developed, however, until now, no unique approach has been presented as a benchmark for extracting natural bioactive compounds from plants. It is widely acknowledged

that the selectivity and efficiency of extraction techniques are contingent on the judicious selection of critical starting parameters, the nature of the plant samples, the structure of the bioactive compounds, and the analytical prowess of the analyst. The present work aims to discuss recent advances in supercritical fluid extraction techniques, especially with carbon dioxide, along with the basic principles of extracting bioactive compounds from natural plant materials such as herbs, spices, aromatic and medicinal plants.

In the second publication (aut. 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) the PhD student presented the results of an investigation into the application of a supercritical CO_2 extraction technique to extract bioactive compounds from *L. album* flowers. The extraction process was conducted at varying temperatures (40, 50, and 60°C) with the utilization of methanol as an additional solvent. This approach was employed to address the limitations of CO_2 extraction, which is known to be ineffective due to its non-polar nature in extracting polar compounds (as correctly identified by the author). The extraction process was carried out at a constant rate of flow, methanol, and pressure. The antioxidant activity of the obtained extracts was determined using three independent methods: DPPH, ABTS and FRAP, as well as the total polyphenol content (TPC) using the Folin-Ciocalteu method. Furthermore, the quantification of polyphenolic compounds in *L. album* flower extracts was conducted by high-performance liquid chromatography coupled to a photodiode array detector (UPLC-PDA), with subsequent confirmation via a triple quadrupole mass detector (TQD). The results obtained demonstrated that the optimal extraction temperature for *L. album* flowers was 50°C, as the extracts exhibited the highest free radical "scavenging" capacity, reducing capacity and total polyphenol content. This finding corroborated the hypothesis that the antioxidant activity and polyphenol content of plant extracts are closely dependent on the extraction conditions employed. The identification of polyphenolic compounds in the obtained extract from *L. album* flowers, using UPLC-PDA, showed that chrysin, pinostrobin, myricetin and trans-3-hydroxycinnamic acid were present in the highest concentrations and may be responsible for the high polyphenol content and antioxidant activity. The categorization of polyphenolic compounds was conducted based on their structural characteristics, namely flavonoids, phenolic acids and phenolic esters, thereby underscoring the heterogeneity of bioactive compounds present in *L. album* flower extracts and their potential for antioxidant and antifungal activities. As the PhD student accurately observed, the findings suggest that SC-CO_2 can be regarded as a viable alternative method for extracting bioactive compounds from *L. album*. The high antioxidant activity and the presence of various bioactive compounds indicate the potential of this plant and the possibility of its application in various industries, including agriculture, food technology, or pharmacy.

However, in the summary for the publication series, it appears on page 12 that extraction was carried out not only under varying temperature parameters, but also under pressure, which is not mentioned either in the publication or in the following section. Please clarify whether pressure was a variable parameter in the experiments? Similarly, on page 16, there is information about the analysis of volatile compounds using GC-MS technique, which is also not mentioned in the publication. Furthermore, the content of these compounds in extracts obtained at elevated pressure (300 bar) and reduced temperature (40°C) is also mentioned. As will be demonstrated subsequently, this variant is unfavorable in comparison to the previously established extraction parameters, i.e. temperature of 50°C and pressure of 250 bar. Furthermore, the antioxidant activity of the extracts obtained in this variant was not checked. On this point, further elucidation from the author would be appreciated.

In the third publication (aut. 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) the efficacy of *L. album* flower extracts in impeding *in vitro* growth and mycotoxin biosynthesis by strains of *Fusarium culmorum* and *proliferatum* was evaluated. The extracts were obtained using a previously optimised supercritical CO_2 extraction method. The *in vitro* antifungal activity of the different concentrations (2.5, 5, 7.5 and 10%) was evaluated using potato dextrose agar (PDA) medium using a technique called "poisoning" by the author. The results demonstrated that *L. album* flower extracts significantly reduced mycelial growth, from 0 to 31% and 28 to 43% for *F. culmorum* and *proliferatum*, respectively. It was observed that higher concentrations of *L. album* extract resulted in a more significant growth inhibition of *F. culmorum*. For *F. proliferatum*, the results indicated a significant inhibitory effect of *L. album* extract at all concentrations tested. A notable reduction in fungal growth was observed at the lowest concentration (2.5%) in comparison to the control, with further inhibition observed at higher concentrations. As the author rightly notes, this suggests that *L. album* extract has strong antifungal properties against *F. proliferatum*, with the inhibitory effect being more pronounced at higher concentrations. The divergent responses observed in *F. culmorum* and *proliferatum* to the application of *L. album* can be attributed to fundamental differences in their genetic composition, metabolic pathways and physiological mechanisms, which is in accordance with existing literature reports. This finding underscores the notion that the efficacy of plant extracts in combating *Fusarium* pathogens is contingent on the characteristics of the plant material in question, the concentration of the extract employed, and the interaction between the extract and the pathogen. The ergosterol (ERG) content of both *F. culmorum* and *proliferatum* was reduced by approximately 89 and 93%, respectively. This decline is presumably attributable to the elevated antifungal activity of the *L. album* extract at elevated concentrations, as previously hypothesised by the PhD student. As the concentration of the extract increases, it more effectively interrupts ERG production or destroys the fungal cell membrane, leading to a decrease in ERG levels. In addition, the synthesis of mycotoxins by both strains was reduced in comparison to the control (PDA without extracts), with the extent of reduction varying according to extract concentration, *Fusarium spp.* type and mycotoxin type. A concentration of 10% was identified as the most effective concentration in reducing mycotoxin biosynthesis in both cases. In conclusion, the study demonstrated that *L. album* flower extracts can inhibit the biosynthesis of well-known mycotoxins and their modified forms and derivatives, which is in agreement with literature reports.

In the fourth publication (aut. 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) the effects of *L. album* flower extracts on seed germination and growth of *F. culmorum*-infected wheat seedlings in pot culture were investigated. The results showed that the phytotoxic effect of the extracts was concentration dependent in both cases. Root length was reduced compared to the control, but root dry weight was not significantly affected by extract application. A slight phytotoxic effect on seed germination was observed, but the antifungal effect on artificially infected wheat seedlings was also confirmed by a reduction in ERG levels and mycotoxin accumulation in roots and leaves after 21 days of inoculation. In contrast, *F. culmorum* DNA was only detected in control samples. In conclusion, the study showed that *L. album* flower extract protects wheat against the pathogen responsible for *Fusarium* crown and root rot,

but as the author rightly points out, further research is needed to investigate the effects of *L. album* extracts on key regulatory genes for mycotoxin biosynthetic pathways.

Publication five (aut. 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) describes a field trial carried out at the Field Experimental Station of Plant Protection – National Research Institute in Winna Góra during the 2022/2023 season to evaluate the potential of using *L. album* flower extract as a foliar spray against mycotoxigenic fungi in two winter wheat varieties: Arcadia and Julius. A previously optimized supercritical CO_2 extraction method was used to obtain *L. album* flower extract. The content of ERG and mycotoxins in the harvested wheat grains was analyzed using dedicated chromatographic methods. The results obtained showed a significant reduction in ERG levels in field plots treated with *L. album* extract, almost 3-fold for the Arkadia and almost 2.5-fold for the Julius. The treatment with *L. album* extract significantly reduced the biosynthesis of mycotoxins (deoxynivalenol (DON) and zearalenone (ZEN)) in both varieties. In addition, the Arcadia showed greater resistance to *Fusarium* infections and the antifungal effect of *L. album* was more pronounced in the Julius, which proved to be more susceptible. In conclusion, *L. album* flower extract showed promising antifungal activity against *F. culmorum* in winter wheat varieties in field trials, suggesting a potential alternative to synthetic fungicides. However, as complete prevention of mycotoxin contamination was not achieved, further research is warranted to optimize extract concentrations and conduct long-term analyses to consider this plant extract as a sustainable control agent.

Discussing the results presented in her thesis, Pascaline Aimee Uwineza, M.Sc., showed that her experiments provided important new insights indicating that *L. album* flower extracts are a promising natural antifungal agent for reducing *Fusarium* infection in wheat. I*n vitro*, *L. album* flower extracts showed significant inhibitory activity against *F. culmorum* and *proliferatum* grown on PDA medium. *In vivo* studies further confirmed the antifungal potential of the extracts by reducing ERG and mycotoxin levels in wheat seedlings, grains and field trials infected with *F. culmorum*. In addition, the thesis highlights the importance of balancing antifungal efficacy with potential phytotoxic effects on crop growth. Overall, it represents an important step towards the development of environmentally friendly and natural alternatives to synthetic fungicides for the control of Fusarium infections in wheat. It can be said to pave the way for further research into natural plant extracts to promote sustainable wheat production and exploit locally available, underutilized plants, thus contributing to the ongoing global discussion on environmentally friendly methods of disease control. And here I would still like to ask the author about the ecological consequences of using *L. album* flower extract.

The PhD student has drawn appropriate conclusions. The research carried out and the conclusions formulated on the basis of it place appropriate emphasis on cognitive and applied significance and are relevant to the objectives set out in the thesis. The only thing I missed was information about further research perspectives, which I would like to ask the PhD student about. Do you plan to continue your research into the optimization of extract formulations for agricultural applications?

The comments contained in this review do not in any way diminish the scientific value of the reviewed work, which is a valuable scientific study that demonstrates the author's good substantive and analytical preparation.

Summary: the publications that make up Pascaline Aimee Uwineza's, M.Sc. doctoral thesis constitute an original contribution to the development of knowledge in the field of food technology and nutrition and are fully in line with the current trend towards interdisciplinary research.

Final evaluation

Summarizing the series of publications constituting the thesis and the accompanying summary, I conclude that Pascaline Aimee Uwineza M.Sc. has demonstrated a good knowledge of the research topic. She has correctly planned and carried out the experiments and analyses, demonstrating her skill in the use of modern analytical apparatus, and has obtained a large number of valuable results.

The scientific achievements of Pascaline Aimee Uwineza M.Sc. includes 12 publications, 11 of which were published in journals indexed in Journal Citation Reports. The PhD student's h-index according to the Scopus database is 7. She has participated as a co-investigator in 1 research project financed by the National Science Centre, Poland and 1 teaching project financed by the National Centre for Research and Development. She has presented 2 papers and 5 posters at national and international scientific conferences, winning an award once. She has completed 3 scientific internships at home and abroad and participated in the Erasmus+ programme.

I conclude that the doctoral thesis submitted for review by Pascaline Aimee Uwineza, M.Sc., entitled. „Use of natural extracts from *Lamium album* in the biological protection of cereals against *Fusarium* pathogens", is an original scientific achievement and meets all the requirements for a thesis of this type as set out in Article 187 of the Act of 20 July 2018 Law on Higher Education and Science. On the basis of the above, I request the Scientific Council of the Discipline of Food Technology and Nutrition of the University of Life Sciences in Poznan to accept the dissertation and admit Pascaline Aimee Uwineza, M.Sc. to further stages of doctoral proceedings.

Magdalena Surma

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