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**Formal basis**

The opinion was prepared for the Council of the Discipline of Biological Sciences at Nicolaus Copernicus University in Toruń, in accordance with the decision adopted at the meeting held on 20 March 2026.

**Review of the Doctoral Dissertation submitted by MSc Matteo Marangi**

**Title of the dissertation:**

**Potential of *Salicornia europaea* in decreasing colonization of plants by  
Human Pathogenic Microorganisms**

**1. Introduction**

The doctoral dissertation of MSc Matteo Marangi was carried out at two following institutions: at the Faculty of Biology and Veterinary Sciences, in the Department of Microbiology at Nicolaus Copernicus University in Toruń under the supervision of Professor Katarzyna Hryniewicz, and at the University of Rostock, Faculty for Agriculture, Civil and Environmental Engineering, under the supervision of Professor Christel Baum.

**2. Evaluation of the Research Topic**

The dissertation addresses an important topic concerning the halophyte *Salicornia europaea* L. (common glasswort), a plant under strict species protection, occurring in saline habitats across Eurasia and northern Africa. In Poland, it is found both along the Baltic coast and in the central part of the country. According to the literature, *Salicornia europaea* L. is an obligatory halophyte (requiring minimal salt concentrations for growth), which gives it a remarkably distinctive flavour and allows its use as a culinary ingredient imparting a unique,

naturally salty character. For this reason, the plant is often referred to as sea asparagus, sea pickle, or sea bean. What is more, *Salicornia* possesses numerous health-promoting properties due to its high content of iodine, potassium, iron, and calcium, as well as polyphenols, vitamin A, and carotene. These characteristics make it a highly compelling and valuable research subject. Therefore, the choice of the research topic, the manner in which it was carried out, as well as the significance and necessity of the results obtained, should be assessed very highly.

### 3. Formal Evaluation

The dissertation consists of eight main, numbered sections (Introduction, Hypotheses, Research Objectives, Materials and Methods, Results, Discussion, Conclusions). In addition, it includes several unnumbered parts: Acknowledgements, Abbreviations, Abstracts (in Polish and English), References, three Appendices, and information about the Author.

The structure of the dissertation is appropriate and fully consistent with the formal guidelines for doctoral theses.

Part of the research results has been published in the journal *Agronomy* in 2024.

### 4. Scholarly Assessment

The title of the doctoral dissertation is appropriate, fully reflects its content, and directly corresponds to the research objectives formulated in the work.

In the evaluated dissertation, the Doctoral Candidate undertook an in-depth investigation of the complex interactions between *Salicornia europaea* L. and three major human pathogenic microorganisms: *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes*. The research encompassed both field experiments and controlled *in vitro* studies. The analyses included the chemical profiling of plant shoots—specifically their lipid and lignin content—as well as the characterization of the saline soil, with particular attention to salinity levels, pH, and colonization by human pathogenic microorganisms (HPMOs), assessed through plant transcriptomics. A further significant component of the study involved the examination of endophytic and rhizosphere bacteria, focusing on their capacity to synthesize microbial volatile organic compounds (mVOCs) with the potential to inhibit the proliferation of pathogenic microorganisms.

In the *Introduction*, the Doctoral Candidate presents the current state of knowledge relevant to the conducted research, drawing upon the most recent and authoritative literature.

This section of the dissertation is characterized by a clear, well-structured, and thoughtfully organized narrative, and is divided into the following six subsections:

- Soil salinization – a pressing contemporary issue
- Revealing the agronomic potential of *Salicornia europaea* as a valuable halophyte
- Human pathogen contamination in crops
- Plant–Human Pathogenic Microorganisms interactions and foodborne illness outbreaks
- The potential role of endophytic and rhizosphere bacteria in Human Pathogenic Microorganisms suppression: microbial volatile organic compounds
- Influence of the chemical composition of plants and soils on endophytes

I have no critical remarks regarding this part of the dissertation. In my assessment, the Introduction is exceptionally clear and provides all essential information necessary to establish an appropriate scientific context for the topic undertaken by the Doctoral Candidate. The selection of literature is likewise appropriate and well-justified.

Next, the Author of the dissertation formulated three main research hypotheses, namely that:

1. the chemical parameters of plants and soils control the abundance of human pathogenic microorganisms and may serve as risk indicators in the context of food safety;
2. the persistence of human pathogenic microorganisms in the studied plant may be limited by the plant's native microbiome and its internal salinity;
3. the inhibition of human pathogenic microorganisms may result from the production of microbial volatile organic compounds by endophytic and rhizosphere bacteria.

The hypotheses are well-conceived; I have only a minor remark regarding the phrasing of hypothesis No. 1. Given that a hypothesis is, by definition, a conjecture requiring verification, it would be more appropriate to state that the chemical parameters of plants and soils *may control* the abundance of human pathogenic microorganisms, rather than that they 'control' it. However, the English version of the hypothesis is phrased correctly.

The Author formulated also three clear research objectives:

1. to quantitatively assess key physicochemical factors—pH, salinity, and lignin content—in both soil and *Salicornia europaea* tissues that influence the establishment and abundance of human pathogenic microorganisms;
2. to investigate the temporal and spatial dynamics of colonization of *Salicornia europaea* by human pathogenic microorganisms and to determine how this interaction affects the plant's gene expression profile;
3. to identify and select endophytic and rhizosphere bacterial strains originating from *Salicornia europaea* that exhibit inhibitory activity against human pathogenic microorganisms.

For the chemical characterization of the studied soils, the Author employed appropriately selected analytical techniques, including atomic absorption spectrometry, ion chromatography, and optical emission spectrometry. The molecular methods were likewise correctly applied and executed, while the analysis of microbial volatile compounds was performed using gas chromatography–mass spectrometry. The dissertation therefore draws upon results generated with state-of-the-art analytical methodologies.

The Doctoral Candidate also deserves recognition for clearly indicating which analyses were commissioned and carried out within institutional collaborations by qualified specialists. It is well understood that no Doctoral Candidate is expected to independently perform all analyses or possess expert-level proficiency in every technique; the ability to engage in thoughtful and effective scientific collaboration is both valuable and commendable.

The experimental work resulted in the analysis of 2,000 plant samples (125 plants across 16 experimental variants), representing a substantial analytical effort. The schematic overview of the experimental design provided on page 31 greatly facilitates the reader's understanding of the study. In addition, the Author examined 58 endophytic and rhizosphere bacterial isolates belonging to four classes: *Bacilli*, *Gammaproteobacteria*, *Actinomycetes*, and *Alphaproteobacteria*. Overall, the *Methods* are well described and sufficiently detailed; the only missing element is information regarding the number of replicates performed for each analysis.

The *Results* are presented carefully, in a logical sequence, and are supported by well-constructed graphical materials. The decision to conduct analyses on two soil types—old and young marshes—was well justified, given the pronounced differences in their chemical properties, such as pH, which is a key determinant of potential microbial community structure and biological activity in soils. The soils also differed in salinity and physical structure, both

of which are important parameters influencing microbial biodiversity. The findings therefore substantiate the conclusion that the dynamics of human pathogenic microorganisms are strongly shaped by local soil conditions.

The *Discussion* part is divided into three subsections, which facilitates the analysis of the individual aspects addressed in the dissertation. The Author begins by comparing his findings on the influence of soil chemistry on the development of human pathogenic microorganisms, correctly emphasizing that saline soils are generally unfavorable for the growth of the examined pathogenic bacteria, as salinity inhibits their proliferation and activity. Subsequently, the Author compared his observations with the available literature concerning the inoculation of plants with human pathogenic microorganisms and their varying effects on plant colonization and growth parameters. As he notes, his findings contrast with observations reported for radish, indicating that inoculation of *Salicornia europaea* with *Escherichia coli* and *Listeria monocytogenes* resulted in an increase in plant biomass, suggesting a potential growth-stimulating effect. In the concluding part of the Discussion, the Doctoral Candidate offers a thoughtful comparison of his findings with the existing—but indeed very limited—body of knowledge on microbial volatile compounds as emerging bioactive agents capable of suppressing foodborne pathogens. This component of the dissertation represents a distinctly novel contribution and meaningfully advances the current state of scientific understanding.

The section entitled *Conclusions* functions primarily as a summary of the obtained results, and should therefore be titled accordingly. The Author directly addresses the three research hypotheses and describes the observations related to each of them. However, the actual conclusions would have benefited from being presented in a structured, bullet-point format.

The final numbered section of the dissertation, *Perspectives for Experimental Validation*, presents the Author's proposals for continuing the research, grounded in his knowledge and experience. This forward-looking approach reflects the Doctoral Candidate's considerable scientific maturity, demonstrates his ability to plan future research, and clearly indicates his motivation for further academic development — which I wholeheartedly wish him.

In summary, the literature review, the formulation of hypotheses and research objectives, as well as the field and laboratory investigations, were all carried out with appropriate methodological rigor. The obtained results are original and innovative, and they have been correctly illustrated and subjected to proper statistical analysis. In doing so, the Doctoral Candidate has demonstrated the ability to synthesize complex scientific problems and to interpret the resulting data accurately and coherently.

## 5. Key Achievements

The properly designed field experiments, and the broad range of analytical and statistical methods, combined with the Doctoral Candidate's solid preparation, enabled the generation of numerous valuable and scientifically significant results that contribute new knowledge to the field.

As a result of the conducted research, the Author demonstrated that:

- there is a potential protective role of lignin, as evidenced by the negative correlations between lignin content in shoots and the abundance of *Salmonella enterica*, as well as between lignin content in soil organic matter and the abundance of *Escherichia coli*;
- there is a negative correlation between plant growth in saline soils and the abundance of human pathogenic microorganisms, which is relevant in the context of food safety;
- dimethyldisulfide (DMDS) emerged as the principal microbial volatile organic compound with potential antimicrobial activity, produced by specific endophytic and rhizosphere bacteria, a finding of considerable importance for biocontrol and improved food safety in agriculture;
- pH and salinity constitute two key controlling factors and risk indicators for the contamination of *Salicornia europaea* by human pathogenic microorganisms;
- the abundance of human pathogenic microorganisms was higher in roots than in shoots, a pattern confirmed both in culture-based analyses and qPCR assays;
- isolates belonging to the classes *Bacilli* and *Actinomycetes* exhibited predominantly inhibitory activity against *Listeria monocytogenes*, with several strains also suppressing the growth of *Escherichia coli*;
- one isolate from the *Alphaproteobacteria* group displayed broad-spectrum inhibitory activity against all three human pathogenic microorganisms examined.

## 6. Comments and Questions

The following comments and questions arise from the evaluation of the dissertation:

- information on the distribution and use of *S. europaea* in Poland is missing — it would be valuable to indicate the scale of its occurrence, where it grows, and whether it is used in Poland, and if so, for what purposes;

- the number of replicates for chemical and microbiological analyses is not specified — although the Reviewer has no doubt that replicates were performed, as the results underwent statistical analysis, this information is absent both in the dissertation and in the related publication;
- the number of replicates used for DNA isolation is also not provided;
- Figure 4 would benefit from the addition of error bars or standard deviation values to improve the clarity of data presentation.
- the caption of Table 2 should be moved to the page on which the table itself appears;
- the captions for Figures 5, 13, 16, and 19 should be placed directly below the figures rather than on the following page.

## 7. Final Conclusion

The doctoral dissertation of MSc Matteo Marangi, entitled “Potential of *Salicornia europaea* in decreasing colonization of plants by Human Pathogenic Microorganisms”, meets the requirements set for doctoral theses by the Act of 20 July 2018 — Law on Higher Education and Science (Journal of Laws, item 1668), as well as the Act of 2 July 2018 — Provisions introducing the Act on Higher Education and Science (Journal of Laws, item 1669).

Mr. Matteo Marangi has demonstrated in the submitted dissertation that he meets all the criteria specified in the aforementioned legislation. Therefore, I recommend that MSc Matteo Marangi be admitted to the subsequent stages of the doctoral procedure.

Furthermore, considering the high scientific value of the dissertation, as well as the relevance and novelty of the research topic, I hereby propose that the doctoral dissertation of MSc Matteo Marangi be awarded a distinction.

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