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Response Reviewer 1:

We sincerely thank Reviewer 1 for the time and effort dedicated to evaluating our manuscript and for providing constructive and insightful comments that have helped us improve its quality. We have carefully addressed all the points raised, revising the text accordingly to enhance its clarity, precision, and scientific rigor. We truly appreciate the reviewer's contribution to refining our work. We hope that this revised version, which also incorporates the modifications suggested by the other reviewers, will now meet the expectations of both the reviewer and the editorial board, and that it will be favourably considered for publication in *Agronomy*.

Comment 1: "The title should be modified so that it is more related to the research work carried out."

Response: We thank the reviewer for this valuable suggestion. The title has been revised to better reflect the scope and objectives of our study, emphasizing the multifunctional nature of the endophytic fungi investigated.
Previous title: *Endophytic fungi from ginger (Zingiber officinale) as potential sources of antimicrobial, enzymatic, and antioxidant metabolites.*
Revised title: *Multifunctional Endophytic Fungi from Ginger (Zingiber officinale) with Antimicrobial, Enzymatic, and Antioxidant Potential.*

Comment 2: "The name of microorganisms must be in cursive."

Response: We appreciate the reviewer's observation. All scientific names of microorganisms have been carefully revised throughout the manuscript and are now consistently formatted in italics.

Comment 3: "Nomenclature of nine endophytes (J34, J35, etc.) is not clear; it could be eliminated."

Response: We thank the reviewer for this comment. The alphanumeric codes (e.g., J34, J35, J47) correspond to internal identifiers assigned to each fungal isolate during the isolation and maintenance process in the culture collection of the University of Cádiz. These codes were sequentially assigned as colonies were isolated from the original ginger tissues and subsequently selected based on their morphological characteristics as filamentous fungi.
To clarify this point, an explanatory sentence has been added in *Section 4.1 (Isolation and Purification of Endophytic Fungi from Ginger Tubers)*. The use of internal codes has been maintained in the revised version to ensure consistency with the results tables and figures and to preserve traceability of the isolates for future reference.

Comment 4: "Table 2, antifungal activity: Are the showed results the mean of two replicates and the standard deviation?"

Response: We thank the reviewer for this valuable observation. We wish to clarify that all experiments presented in this study —including antifungal, antibacterial, enzymatic, and antioxidant assays— were performed **in triplicate**, and the values reported in Table 2 correspond to the **mean ± standard deviation of three independent replicates**.
The mention of "two replicates" in the previous version of the manuscript was a **typographical error** introduced during translation and editing. This has now been corrected. To prevent further misunderstanding, we have added the following clarification in *Section 4.4 (Antagonistic Activity Assays)*:
"The antagonistic activity assays, showed in this section 4, were performed in trip-licate, and the inhibition values were expressed as the mean ± standard deviation of three independent experiments to ensure statistical reliability and reproducibility of the results."
Furthermore, our group systematically performs microbiological and biochemical assays in triplicate,

as evidenced by our previous MDPI publications, where the same methodological standard was applied:

– **Bolívar-Anillo, H.J.; Izquierdo-Bueno, I.; González-Rey, E.; González-Rodríguez, V.E.; Cantoral, J.M.; Collado, I.G.; Garrido, C.** *In Vitro Analysis of the Antagonistic Biological and Chemical Interactions between the Endophyte* **Sordaria tomento-alba** *and the Phytopathogen* **Botrytis cinerea**. *Int. J. Mol. Sci.* **2024**, *25*, 1022. https://doi.org/10.3390/ijms25021022.

– **Bódalo, A.; Borrego, R.; Garrido, C.; Bolivar-Anillo, H.J.; Cantoral, J.M.; Vela-Delgado, M.D.; González-Rodríguez, V.E.; Carbú, M.** *In Vitro Studies of Endophytic Bacteria Isolated from Ginger (Zingiber officinale) as Potential Plant-Growth-Promoting and Biocontrol Agents against* **Botrytis cinerea** *and* **Colletotrichum acutatum**. *Plants* **2023**, *12*, 4032. https://doi.org/10.3390/plants12234032.

– **Bolivar-Anillo, H.J.; González-Rodríguez, V.E.; Cantoral, J.M.; García-Sánchez, D.; Collado, I.G.; Garrido, C.** *Endophytic Bacteria* **Bacillus subtilis**, *Isolated from* **Zea mays**, *as Potential Biocontrol Agent against* **Botrytis cinerea**. *Biology* **2021**, *10*, 492. https://doi.org/10.3390/biology10060492

Comment 5: "The results shown in Table 3 are not understood; Table 3 must be eliminated as well as the methodology and result description related with this experiment."

Response: We thank the reviewer for this observation. In the revised version of the manuscript, Table 3 has been completely reformatted and updated to include quantitative data derived from three independent replicates, expressed as mean ± standard deviation.
This modification enhances the clarity and statistical robustness of the results, allowing a clearer comparison of the antibacterial activity exhibited by the isolated fungi against the different bacterial strains tested.
We have retained this experiment because it provides valuable information demonstrating that the endophytic fungi isolated and identified in this study display differential antimicrobial activity, showing inhibition against one bacterial species but not against another.
The corresponding sections of the *Materials and Methods* and *Results* have been revised accordingly to describe the quantitative approach and the inclusion of standard deviation values.

Comment 6: "Table 6: concentration of the extracts must be indicated."

Response: We thank the reviewer for this valuable observation. In the revised version of the manuscript, the concentration of the fungal extracts used in all assays has been clearly indicated both in Table 6 and in the corresponding section of *Materials and Methods*.
All crude extracts were adjusted to a final concentration of 1 mg/mL of total metabolites prior to testing, ensuring consistency and comparability among samples. This clarification enhances the transparency and reproducibility of the experimental procedures.

Comment 7: *"*The Discussion section is too general and should be improved by avoiding ambiguous statements and irrelevant information. It should focus more directly on the results and their interpretation."

Response: We thank the reviewer for this valuable observation. The Discussion section has been thoroughly revised to remove ambiguous or overly general statements and to focus more clearly on the interpretation of our experimental results. The text was streamlined and reorganized to reinforce the connection between the findings and their biological and biotechnological significance. Transitions between subsections were also improved to ensure a coherent and data-driven narrative.

Furthermore, to maintain full consistency throughout the manuscript, the Abstract and Conclusions sections were also reformulated in accordance with these revisions. Both now summarize the main quantitative results and highlight the key findings more clearly, ensuring overall coherence and clarity in the presentation of the study.

We sincerely thank the reviewer for their constructive feedback. All comments have been carefully addressed, and the manuscript has been revised accordingly to enhance its clarity, rigor, and overall scientific quality. We hope that the revised version will now meet the reviewer's expectations and be suitable for publication in Agronomy.

Sincerely,

Dr. Carlos Garrido
Laboratorio de Microbiología
Facultad de Ciencias
Universidad de Cádiz