Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

PROGRAMA DE DOCTORADO EN CIENCIA E INGENIERÍA AGROALIMENTARIA Y DE BIOSISTEMAS

TESIS DOCTORAL

NUEVAS FORMULACIONES ANTIMICROBIANAS BASADAS EN EXTRACTOS VEGETALES CON APLICACIONES EN EL ÁMBITO AGROFORESTAL

Presentada por Eva Sánchez Hernández para optar al grado de Doctora por la Universidad de Valladolid

Dirigida por:
Pablo Martín Ramos

Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

DOCTORAL PROGRAM IN AGRI-FOOD AND BIOSYSTEMS SCIENCE AND ENGINEERING

DOCTORAL THESIS

NOVEL ANTIMICROBIAL FORMULATIONS BASED ON PLANT EXTRACTS FOR AGROFORESTRY APPLICATIONS

Submitted by Eva Sánchez-Hernández to obtain the degree of Doctor from the University of Valladolid

Supervised by:
Pablo Martín Ramos

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I, Dr. Pablo Martín Ramos, Associate Professor in the Department of Agroforestry Engineering at the University of Valladolid,

CERTIFY:

That the present Thesis, titled "**NOVEL ANTIMICROBIAL FORMULATIONS BASED ON PLANT EXTRACTS FOR AGROFORESTRY APPLICATIONS**", corresponding to the research plan approved by the Doctoral Committee of the Doctoral Program in Agricultural and Biosystems Science and Engineering, and organized as a compilation of publications to obtain the Doctoral degree from the University of Valladolid, has been carried out under my supervision by Ms. Eva Sánchez Hernández at the Higher Technical School of Agricultural Engineering at the University of Valladolid. It has my authorization to be presented and to undergo the necessary procedures until its evaluation by the respective Committee.

For the record, I sign this certificate in Palencia, on November 27, 2023.

Firmado digitalmente por MARTIN RAMOS PABLO - 71128357Y

Dr. Pablo Martín Ramos

REPORT ON THE PUBLICATIONS INCLUDED IN THIS DOCTORAL THESIS

This Doctoral Thesis comprises a compilation of previously published works, consisting of nineteen articles published in scientific journals indexed in the Web of Science (WOS) – Journal Citation Reports (JCR) database. Seventeen of these journals are in the first quartile (Q1), while two are in the second quartile (Q2). Additionally, this Doctoral Thesis includes three patents, one of which has a PCT (Patent Cooperation Treaty) application. Ms. Eva Sánchez Hernández is the first author in fifteen of the publications (thirteen in Q1 and two in Q2), the second author in four publications, and a co-inventor of the three patents. The University of Valladolid owns the rights to these patents. The complete references for the articles, grouped by thematic area (woody crops, cereal crops, horticultural crops, post-harvest protection) for ease of reference, and patents that constitute the body of the Doctoral Thesis are as follows:

Woody crops

1. E. Sánchez Hernández, L. Buzón Durán, N. Langa Lomba, J. Casanova Gascón, B. Lorenzo Vidal, J. Martín Gil, P. Martín-Ramos. "Characterization and antimicrobial activity of a halophyte from the Asturian coast (Spain): *Limonium binervosum* (G.E.Sm.) C.E.Salmon". *Plants*, 2021, 10(9), 1852, https://doi.org/10.3390/plants10091852; Q1 JCR (Science Edition - Plant Sciences, 39/238). JIF_{2021} = 4.658.
2. E. Sánchez Hernández, L. Buzón Durán, B. Lorenzo Vidal, J. Martín Gil, P. Martín Ramos. "Physicochemical characterization and antimicrobial activity against *Erwinia amylovora*, *Erwinia vitivora*, and *Diplodia seriata* of a light purple *Hibiscus syriacus* L. cultivar". *Plants*, 2021, 10(9), 1876, https://doi.org/10.3390/plants10091876; Q1 JCR (Science Edition - Plant Sciences, 39/238). JIF_{2021} = 4.658.
3. D. Ruano Rosa, E. Sánchez Hernández, R. Baquero Foz, P. Martín Ramos, J. Martín Gil, S. Torres Sánchez, J. Casanova Gascón. "Chitosan-based bioactive formulations for the control of powdery mildew in viticulture". *Agronomy*, 2022, 12(2), 495, https://doi.org/10.3390/agronomy12020495; Q1 JCR (Science Edition – Agronomy, 16/88). JIF_{2022} = 3.7.
4. E. Sánchez Hernández, L. Buzón Durán, J.A. Cuchí Oterino, J. Martín Gil, B. Lorenzo Vidal, P. Martín Ramos. "Dwarf pomegranate (*Punica granatum* L. var. *nana*): Source of 5-HMF and bioactive compounds with applications in the protection of woody crops". *Plants*, 2022, 11(4), 550, https://doi.org/10.3390/plants11040550; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.3.
5. E. Sánchez Hernández, C. Andrés Juan, L. Buzón Durán, A. Correa Guimaraes, J. Martín Gil, Pa. Martín Ramos. "Antifungal activity of methylxanthines against grapevine trunk diseases". *Agronomy*, 2022, 12(4), 885, https://doi.org/10.3390/agronomy12040885; Q1 JCR (Science Edition – Agronomy, 16/88). JIF_{2022} = 3.7.
6. E. Sánchez Hernández, Vicente González García, José Casanova Gascón, Juan J. Barriuso Vargas, Joaquín Balduque Gil, Belén Lorenzo Vidal, Jesús Martín Gil, Pablo Martín-Ramos. "Valorization of *Quercus suber* L. bark as a source of phytochemicals with antimicrobial activity against apple tree diseases". *Plants*, 2022, 11(24), 3415, https://doi.org/10.3390/plants11243415; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.5.
7. E. Sánchez Hernández, J. Balduque Gil, V. González García, J.J. Barriuso Vargas, J. Casanova Gascón, J. Martín Gil, P. Martín Ramos. "Phytochemical profiling of *Sambucus nigra* L. flower and leaf extracts and their antimicrobial potential against almond tree pathogens". *International Journal of Molecular Sciences*, 2023, 24(2), 1154, https://doi.org/10.3390/ijms24021154; Q1 JCR (Science Edition - Biochemistry & Molecular Biology, 66/285). JIF_{2022} = 5.6.

8. E. Sánchez Hernández, J. Balduque Gil, J.J. Barriuso Vargas, J. Casanova Gascón, V. González García, J.A. Cuchí Oterino, B. Lorenzo Vidal, J. Martín Gil, P. Martín Ramos. "Holm oak (*Quercus ilex* subsp. *Ballota* (Desf.) Samp.) bark aqueous ammonia extract for the control of invasive forest pathogens". *International Journal of Molecular Sciences*, 2022, 23(19), 11882, https://doi.org/10.3390/ijms231911882; Q1 JCR (Science Edition - Biochemistry & Molecular Biology, 66/285). JIF_{2022} = 5.6.
9. E. Sánchez Hernández, A. Teixeira, C. Pereira, A. Cruz, J. Martín Gil, R. Oliveira, P. Martín Ramos. "Chemical constituents and antimicrobial activity of a *Ganoderma lucidum* (Curtis.) P. Karst. aqueous ammonia extract". *Plants*, 2023, 12(12), 2271, https://doi.org/10.3390/plants12122271; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.5.

Cereal crops

10. E. Sánchez Hernández, V. González García, A. Correa Guimarães, J. Casanova Gascón, J. Martín Gil, P. Martín Ramos. "Phytochemical profile and activity against *Fusarium* species of *Tamarix gallica* bark aqueous ammonia extract". *Agronomy*, 2023, 13(2), 496, https://doi.org/10.3390/agronomy13020496; Q1 JCR (Science Edition – Agronomy, 16/88). JIF_{2022} = 3.7.

Horticultural crops

11. E. Sánchez Hernández, V. González García, J. Martín Gil, B. Lorenzo Vidal, A. Palacio Bielsa, P. Martín Ramos. "Phytochemical screening and antibacterial activity of *Taxus baccata* L. against *Pectobacterium* spp. and *Dickeya chrysanthemi*". *Horticulturae*, 2023, 9(2), 201, https://doi.org/10.3390/horticulturae9020201; Q1 JCR (Science Edition – Horticulture, 6/36). JIF_{2022} = 3.1.
12. E. Sánchez Hernández, V. González García, A. Palacio Bielsa, B. Lorenzo Vidal, L. Buzón Durán, J. Martín Gil, P. Martín Ramos. "Antibacterial activity of *Ginkgo biloba* extracts against *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas* spp., and *Xanthomonas vesicatoria*". *Horticulturae*, 2023, 9(4), 461, https://doi.org/10.3390/horticulturae9040461; Q1 JCR (Science Edition – Horticulture, 6/36). JIF_{2022} = 3.1.
13. E. Sánchez Hernández, V. González García, A. Palacio Bielsa, J. Casanova Gascón, L.M. Navas Gracia, J. Martín Gil, P. Martín Ramos. "Phytochemical constituents and antimicrobial activity of *Euphorbia serrata* L. extracts for *Borago officinalis* L. crop protection". *Horticulturae*, 2023, 9(6), 652, https://doi.org/10.3390/horticulturae9060652; Q1 JCR (Science Edition – Horticulture, 6/36). JIF_{2022} = 3.1.

Post-harvest protection

14. E. Sánchez Hernández, P. Martín Ramos, J. Martín Gil, A. Santiago Aliste, S. Hernández Navarro, R. Oliveira, V. González García. "*Uncaria tomentosa* L. for the control of strawberry phytopathogens". *Horticulturae*, 2022, 8(8), 672, https://doi.org/10.3390/horticulturae8080672; Q1 JCR (Science Edition – Horticulture, 6/36). JIF_{2022} = 3.1.
15. A. Teixeira, E. Sánchez Hernández, J. Noversa, A. Cunha, I. Cortez, G. Marques, P. Martín Ramos, R. Oliveira. "Antifungal activity of plant waste extracts against phytopathogenic fungi: *Allium sativum* peels extract as a promising product targeting the fungal plasma membrane and cell wall". *Horticulturae*, 2023, 9(2), 136, https://doi.org/10.3390/horticulturae9020136; Q1 JCR (Science Edition – Horticulture, 6/66). JIF_{2022} = 3.1.
16. L. Buzón Durán, E. Sánchez Hernández, M. Sánchez Báscones, M.C. García González, S. Hernández Navarro, A. Correa Guimarães, P. Martín Ramos. "A coating based on bioactive compounds from *Streptomyces* spp. and chitosan oligomers to control *Botrytis cinerea* preserves the quality and improves the shelf life of table grapes". *Plants*, 2023, 12(3), 577,

https://doi.org/10.3390/plants12030577; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.5.

17. E. Sánchez Hernández, P. Martín Ramos, L.M. Navas Gracia, J. Martín Gil, A. Garcés Claver, A. Flores León, V. González García. "*Armeria maritima* (Mill.) Willd. flower hydromethanolic extract for Cucurbitaceae fungal diseases control". *Molecules*, 2023, 28(9), 3730, https://doi.org/10.3390/molecules28093730; Q2 JCR (Science Edition - Chemistry, Multidisciplinary, 63/178). JIF_{2022} = 4.6.
18. L. Buzón Durán, E. Sánchez Hernández, P. Martín Ramos, L.M. Navas Gracia, M.C. García González, R. Oliveira, J. Martín Gil. "*Silene uniflora* extracts for strawberry postharvest protection". *Plants*, 2023, 12(9), 1846, https://doi.org/10.3390/plants12091846; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.5.
19. E. Sánchez Hernández, J. Álvarez Martínez, V. González García, J. Casanova Gascón, J. Martín Gil, P. Martín Ramos. "*Helichrysum stoechas* (L.) Moench inflorescence extract for tomato disease management". *Molecules*, 2023, 28(15), 5861, https://doi.org/10.3390/molecules28155861; Q2 JCR (Science Edition - Chemistry, Multidisciplinary, 63/178). JIF_{2022} = 4.6.

Patents

20. E. Sánchez Hernández, J. Martín Gil, L. Buzón Durán, P. Martín Ramos. "Compuesto reticulado de lignina metacrilada y oligómeros de quitosano capaz de actuar como nanotransportador de compuestos bioactivos, método de obtención y usos". Spanish patent with application number P202131019, filed on 29th October 2021. Granted on 3rd May 2023, with patent number ES2940132.
21. E. Sánchez Hernández, A. Santiago Aliste, Je. Martín Gil, P. Martín Ramos. "Nanomaterial basado en el autoensamblaje de g-C_3N_4 y oligómeros de quitosano, proceso de obtención y usos". Spanish patent with application number P202230668, filed on 20th July 2022. Extended with international application number PCT/ES2023/070409.
22. E. Sánchez Hernández, A. Santiago Aliste, J. Martín Gil, P. Martín Ramos. "Nanomaterial encapsulante formado por g-C_3N_4 y oligómeros de quitosano enlazados a hidroxiapatito, proceso de obtención y usos". Spanish patent with application number P202330435, filed on 31st May 2023.

Every article featured in this Doctoral Thesis has been published through the Open Access (OA) model, ensuring that there are no financial burdens on readers, authors, institutions, or associated research projects. This achievement has been facilitated by full waivers generously provided by the Open Access (OA) publisher, mirroring the principles of Diamond Open Access. This approach is in harmony with the guidelines set forth in the San Francisco Declaration on Research Assessment (DORA) and the Coalition for Advancing Research Assessment (CoARA).

Article no. 4 has been honored with the designation of "*Editor's Choice*". Article no. 11 has been distinguished as a "*Feature Paper*". Article 10 has been distinguished as a "*Feature Paper*". Article no. 12 has been selected as the "*Journal title story*" in *Horticulturae* (15/05/2023). It is prominently featured on the main page of the journal in the "*Highly accessed articles*" section due to the significant interest it has generated, evidenced by a high number of downloads since its publication on 05/04/2023. Additionally, it has been chosen as "*Editor's Choice*". Article no. 13 has been recognized as a "*Feature Paper*" and selected as "*Editor's Choice*". Article no. 14 has also been recognized as a "*Feature paper*" and selected as "*Editor's Choice*" for the July-December 2022 period in the category of "*Postharvest Biology, Quality, Safety, and Technology*". Article no. 15 has been chosen as the cover for the February issue of the journal, standing out among 166 articles published in that edition. It has also been acknowledged as a "*Feature Paper*".

Other articles in JCR-indexed journals, co-authored during the development of the Doctoral Thesis and related to the previous ones, have been excluded from the compendium because they are part of other previously defended Doctoral Theses:

23. E. Sánchez Hernández, L. Buzón Durán, C. Andrés Juan, B. Lorenzo Vidal, J. Martín Gil, P. Martín-Ramos. "Physicochemical characterization of *Crithmum maritimum* L. and *Daucus carota* subsp. *gummifer* (Syme) Hook.fil. and their antimicrobial activity against apple tree and grapevine phytopathogens". *Agronomy*, 2021, 11(5), 886, https://doi.org/10.3390/agronomy11050886; Q1 JCR (Science Edition – Agronomy, 18/90). JIF_{2021} = 3.949.
24. N. Langa Lomba, L. Buzón Durán, P. Martín Ramos, J. Casanova Gascón, J. Martín Gil, E. Sánchez Hernández, V. González García. "Assessment of conjugate complexes of chitosan and *Urtica dioica* or *Equisetum arvense* extracts for the control of grapevine trunk pathogens". *Agronomy*, 2021, 11(5), 976; https://doi.org/10.3390/agronomy11050976; Q1 JCR (Science Edition – Agronomy, 18/90). JIF_{2021} = 3.949.
25. N. Langa Lomba, L. Buzón Durán, E. Sánchez Hernández, P. Martín Ramos, J. Casanova Gascón, J. Martín Gil, V. González García. "Antifungal activity against *Botryosphaeriaceae* fungi of the hydro-methanolic extract of *Silybum marianum* capitula conjugated with stevioside". *Plants*, 2021, 10(7), 1363, https://doi.org/10.3390/plants10071363; Q1 JCR (Science Edition - Plant Sciences, 39/238). JIF_{2021} = 4.658.
26. N. Langa Lomba, E. Sánchez Hernández, L. Buzón Durán, V. González García, J. Casanova Gascón, J. Martín Gil, P. Martín Ramos. "Activity of anthracenediones and flavoring phenols in hydromethanolic extracts of *Rubia tinctorum* against grapevine phytopathogenic fungi". *Plants*, 2021, 10(8), 1527; https://doi:10.3390/plants10081527; Q1 JCR (Science Edition - Plant Sciences, 39/238). JIF_{2021} = 4.658.
27. E. Sánchez Hernández, N. Langa Lomba, V. González García, J. Casanova Gascón, J. Martín Gil, A. Santiago Aliste, S. Torres Sánchez, P. Martín Ramos. "Lignin-chitosan nanocarriers for the delivery of bioactive natural products against wood-decay phytopathogens". *Agronomy*, 2022, 12(2), 461; https://doi.org/10.3390/agronomy12020461; Q1 JCR (Science Edition – Agronomy, 16/88), JIF_{2022} = 3.7.
28. L. Buzón Durán, N. Langa Lomba, V. González García, J. Casanova Gascón, E. Sánchez Hernández, J. Martín Gil, P. Martín Ramos. "Rutin-stevioside and related conjugates for potential control of grapevine trunk diseases". *Phytopathologia Mediterranea*, 2022, 61 (1), 65, https://doi.org/10.36253/phyto-13108; Q2 JCR (Science Edition - Plant Sciences, 30/88). JIF_{2022} = 2.4.
29. A. Santiago Aliste, E. Sánchez Hernández, N. Langa Lomba, V. González García, J. Casanova Gascón, J. Martín Gil, P. Martín Ramos. "Multifunctional nanocarriers based on chitosan oligomers and graphitic carbon nitride assembly". *Materials*, 2022, 15(24), 8981; https://doi.org/10.3390/ma15248981; Q2 JCR (Science Edition - Metallurgy & Metallurgical Engineering, 20/78), JIF_{2022} = 3.4.
30. N. Langa Lomba, J. Grimplet, E. Sánchez Hernández, P. Martín Ramos, J. Casanova Gascón, C. Julián Lagunas, V. González García. "Metagenomic study of fungal microbial communities in two PDO Somontano vineyards (Huesca, Spain): Effects of age, plant genotype, and initial phytosanitary status on the priming and selection of their associated microorganisms" *Plants*, 2023, 12(12), 2251, https://doi.org/10.3390/plants12122251; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.5.
31. A. Santiago Aliste, E. Sánchez Hernández, L. Buzón Durán, J.L. Marcos Robles, J. Martín Gil, P. Martín Ramos. "*Uncaria tomentosa*-loaded chitosan oligomers–hydroxyapatite–carbon nitride nanocarriers for postharvest fruit protection". *Agronomy*, 2023, 13(9), 2189; https://doi.org/10.3390/agronomy13092189; Q1 JCR (Science Edition – Agronomy, 16/88), JIF_{2022} = 3.7.

In addition, other articles published in JCR-indexed journals, co-authored during the development of the Doctoral Thesis and related to the aforementioned ones, have not been included in the Doctoral Thesis compendium for reasons of thematic coherence:

32. B. Ayuda Durán, E. Sánchez Hernández, S. González Manzano, C. Santos Buelga, A.M. González Paramás. "The effects of polyphenols against oxidative stress in *Caenorhabditis elegans* are determined by coexisting bacteria". *Frontiers in Nutrition*, 2022, 9, 989427, https://doi.org/10.3389/fnut.2022.989427; Q2 JCR (Nutrition & Dietetics, 28/88). JIF_{2022} = 5.0.
33. J. Balduque Gil, F.J. Lacueva Pérez, G. Labata Lezaun, R. del Hoyo Alonso, S. Ilarri, E. Sánchez Hernández, P. Martín Ramos, J.J. Barriuso Vargas. "Big data and machine learning to improve European grapevine moth (*Lobesia botrana*) predictions". *Plants*, 2023, 12 (3), 633, https://doi.org/10.3390/plants12030633; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.5.
34. A. Santiago Aliste, E. Sánchez Hernández, C. Andrés Juan, P. Chamorro Posada, G. Antorrena, J. Martín Gil, P. Martín Ramos. "F,O,S-codoped graphitic carbon nitride as an efficient photocatalyst for the synthesis of benzoxazoles and benzimidazoles". *Catalysts*, 2023, 13(2), 385; https://doi.org/10.3390/catal13020385; Q2 JCR (Science Edition - Chemistry, Physical, 71/161). JIF_{2022} = 3.9.
35. E. Sánchez Hernández, P. Martín Ramos, J. Niño Sánchez, S. Diez Hermano, F. Álvarez Taboada, R. Pérez García, A. Santiago Aliste, J. Martín Gil, J.J. Diez Casero. "Characterization of *Leptoglossus occidentalis* eggs and egg glue". *Insects*, 2023, 14 (4), 396, https://doi.org/10.3390/insects14040396; Q1 JCR (Science Edition – Entomology, 15/100). JIF_{2022} = 3.0.

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En memoria de mi abuela Antonia,
por su crianza y amor a las plantas

A science – so the Savans say,
"Comparative Anatomy" –
By which a single bone –
Is made a secret to unfold
Of some rare tenant of the mold–
Else perished in the stone –

So to the eye prospective led,
This meekest flower of the mead
Opon a winter's day,
Stands representative in gold
Of Rose and Lily, manifold,
And countless Butterfly!

Emily Dickinson, poem 147 (Emily Dickinson, *Poemas y Cartas 1–600*, trad. A. Mañeru Méndez y C. Oliart Delgado de Torres, eds. A. Mañeru Méndez y C. Oliart Delgado de Torres, Madrid, Sabina editorial, 2023).

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To "Discoteros UF," for rekindling my interest in Ultimate Frisbee, and turning those moments into a place of relaxation, sports, and camaraderie.

RESUMEN

En el contexto actual, caracterizado por el aumento de la población mundial y el cambio climático, la prioridad de asegurar la productividad y calidad de los cultivos agrícolas ha sido ampliamente reconocida a nivel internacional, especialmente a través del Objetivo de Desarrollo Sostenible (ODS) 2 de la Agenda 2030. No obstante, es crucial abordar este desafío de manera responsable y sostenible, como subraya el ODS 12, particularmente en lo que respecta a la substitución de productos fitosanitarios convencionales. La presente Tesis Doctoral se centra en la aplicación de extractos naturales para el control de fitopatógenos, tanto en la fase de precosecha como en la de postcosecha. El énfasis recae en la extracción y caracterización físicoquímica de una variedad de extractos, abarcando plantas como *Limonium binervosum, Hibiscus syriacus, Punica granatum* var. *nana, Quercus suber, Sambucus nigra, Quercus ilex* subsp. *ballota, Tamarix gallica, Taxus baccata, Ginkgo biloba, Euphorbia serrata, Uncaria tomentosa, Armeria maritima, Silene uniflora, Helichrysum stoechas*, residuos como las pieles de *Allium sativum*, hongos como *Ganoderma lucidum*, metabolitos secundarios de *Streptomyces* spp., y productos naturales purificados como las metilxantinas. La eficacia de estos extractos se evaluó frente a patógenos que afectan a cultivos leñosos, extensivos (cereales) y hortícolas, así como patógenos postcosecha, mediante ensayos *in vitro, in vivo* y *ex situ*. Los resultados obtenidos revelan que las eficacias de estos extractos son comparables o incluso superiores a las de fitosanitarios convencionales como las estrobirulinas o los fosfonatos. Este enfoque no solo demuestra ser prometedor en términos de control de plagas, sino que también resalta la viabilidad de adoptar prácticas agrícolas más sostenibles y respetuosas con el medio ambiente, alineándose con los principios establecidos en la Agenda 2030.

Palabras clave: biorracionales, fitopatología, quitosano, productos naturales, protección de cultivos, protección postcosecha.

ABSTRACT

In the current context, characterized by the increasing global population and climate change, the priority of ensuring productivity and quality in agricultural crops has been widely recognized internationally, particularly through Sustainable Development Goal (SDG) 2 of the 2030 Agenda. However, it is crucial to address this challenge responsibly and sustainably, as emphasized by SDG 12, especially regarding the substitution of conventional phytosanitary products. This Doctoral Thesis focuses on the application of natural extracts for the control of phytopathogens, both in the pre-harvest and post-harvest phases. The emphasis is on the extraction and physicochemical characterization of a variety of extracts, including plants such as *Limonium binervosum, Hibiscus syriacus, Punica granatum* var. *nana, Quercus suber, Sambucus nigra, Quercus ilex* subsp. *ballota, Tamarix gallica, Taxus baccata, Ginkgo biloba, Euphorbia serrata, Uncaria tomentosa, Armeria maritima, Silene uniflora, Helichrysum stoechas*, residues like *Allium sativum* peels, fungi such as *Ganoderma lucidum*, secondary metabolites from *Streptomyces* spp., and purified natural products like methylxanthines. The efficacy of these extracts was assessed against pathogens affecting woody, extensive (cereals), and horticultural crops, as well as post-harvest pathogens, through *in vitro, in vivo*, and *ex-situ* trials. The results obtained reveal that the efficacy of these extracts is comparable or even superior to conventional phytosanitary products such as strobilurins or phosphonates. This approach not only proves promising in terms of pest control but also underscores the feasibility of adopting more sustainable and environmentally friendly agricultural practices, aligning with the principles outlined in the 2030 Agenda.

Keywords: biorationals, chitosan, crop protection, natural products, phytopathology, postharvest protection.

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1. INTRODUCTION

1.1. Towards sustainable agriculture

Given the projection of continued global population growth, estimated to reach 9.7 billion people by 2050, a significant increase in food supply is required [1]. This escalation is pivotal for addressing sustainability and global food security, aligning with Sustainable Development Goal (SDG) 2 of the 2030 Agenda. Currently, approximately 40% of the world's food production is lost due to pathogens, pests, and weeds, posing the most urgent threat to global food security [2]. This results in severe economic and nutritional losses throughout the product value chain [3]. To address this growing demand for food, coupled with the limited availability of additional agricultural land, the primary focus lies on increasing yield per unit area and reducing yield losses [4,5]. Consequently, a further enhancement of plant disease control efficiency is expected to significantly contribute to the global increase in food demand [6]. To ensure sustainable production patterns (SDG target 12.4) and bolster food security, current legislative frameworks advocate for the use of integrated pest management (IPM).

Current plant disease management primarily involves non-chemical measures, which are predominantly preventive and include cultural practices such as utilizing disease-resistant plant cultivars and crop rotation [7]. Chemical pesticides, on the other hand, are used for both preventive and curative disease management [7,8]. They play a crucial role in contemporary agriculture concerning increasing crop yield and quality, enhancing food safety (e.g., in relation to reducing microbial toxins), and improving shelf life [9].

Despite their undeniable positive contribution to effective plant disease and pest control, growing concern surrounds the negative effects of chemical pesticides on human health and their adverse impact on the environment, including soil and water pollution and toxicity to beneficial organisms [10]. Furthermore, intensive pesticide use has led to the development of resistant pests and pathogens, such as fungi insensitive to broad-spectrum strobilurin or azole-based fungicides [11]. This results in even higher application doses and an urgency for alternative pesticides with a different mode of action [12]. However, the availability and variety of these novel pesticides are currently limited and insufficient to counteract the issue of growing resistance development [13,14].

Most developed countries enforce strict regulations on the use of toxic chemical fertilizers and pesticides or outright prohibit them, implementing various integrated pest management programs [15]. Additionally, the accessibility of efficient pesticides is further decreasing due to increasingly stringent legislation [13]. European Union (EU) directives like 91/414/EEC, for instance, resulted in the withdrawal of approximately 750 out of 1,000 products from the legally authorized active pesticide substance list between 1993 and 2011, with only 180 new products registered [16]. In addition to its stricter regulations on pesticide use, the EU advocates for disease management with reduced pesticide inputs, as evidenced by the IPM program implemented through Directive 2009/128/EC. Article 14 of this directive establishes the foundation for sustainable pesticide use, with a primary focus on reducing their usage as a fundamental objective. In Spanish legislation, the sustainable use of phytosanitary products is referenced in Royal Decree 1311/2012.

The development of bioactive natural products for the creation of ecological phytosanitary products is garnering increasing attention as an alternative to synthetic fungicides for managing various plant fungal diseases [17,18]. This is due to their advantages in terms of safety, easy biodegradability, environmental friendliness, low toxicity, and specificity [19]. Furthermore, it is an ecological and cost-effective process without the formation of metabolites after action [20].

The use of bioactive products in crop protection or post-harvest protection has been extensively discussed in several recent reviews [2,21-23]. Additionally, to explore synergies, it is recommended to use bioactive natural products in combination with other substances (such as chitosan) for effective control and to minimize pathogen tolerance [24]. Chitosan exhibits antimicrobial properties but also functions as an elicitor, stimulating natural defense mechanisms [25]. The accepted and potential mechanisms behind its antimicrobial properties are extensively discussed in the review by Ma *et al.* [26]. According to SANCO/12388/2013, chitosan can be used in an aqueous solution for application in various crops, including "berry fruits and small fruits".

1.2. Pathogens of interest in agroforestry

The outbreaks of emerging plant diseases and pests impact food security, national security, and human health, with severe economic implications for agriculture. Emerging plant diseases are already becoming more prevalent, and —in the coming decades— changes in the geographical distribution of pests and pathogens in response to climate change and increased global trade are expected to make them more frequent and severe [27].

This Doctoral Thesis has addressed relevant phytopathogens, both at the Iberian Peninsula and global levels, divided into four major thematic areas: woody crops, horticultural crops, cereal crops, and postharvest fruit protection. A brief discussion of their importance is provided below.

In the context of woody crops, particular attention has been given to the protection of fruit crops, especially in the *Rosaceae* family (*Malus* spp., *Prunus* spp., and *Pyrus* spp.), and viticulture (*Vitis vinifera* L.). Concerning the former, fungi of the genus *Monilinia*, especially *Monilinia fructigena* (Pers.) Honey and *Monilinia laxa* (Aderh. & Ruhland) Honey, cause brown rot disease in stone and pome fruits. In susceptible cultivars, these taxa spread to both young shoots and floral buds, causing cankers on twigs and wilting of growing shoots, as well as fruit rot. *Monilinia laxa* leads to significant losses in stone fruit in the field and post-harvest [28,29]. *Diaporthe amygdali* (Delacr.) Udayanga, Crous, and K.D. Hyde has been identified as the causal agent of canker and branch dieback in almonds and peaches, being associated with fruit rot in peaches and “canker *Fusicoccum*” in almonds [30].

In cultivars of *Prunus spp.* and *Malus spp.*, more than ten species of *Phytophthora* causing root rot, crown rot, and stem and scaffold cankers have been identified. Among them, soilborne infection caused by *Phytophthora megasperma* Drechs. is frequently associated with root and crown rot, as well as trunk cankers [31], leading to tree losses, especially in young plants in poorly drained soils. Meanwhile, *Phytophthora cactorum* (Lebert & Cohn) J. Schröt. causes fruit rot, starting with wilting and culminating in tissue destruction, affecting apple, apricot, citrus, plum, and strawberry crops.

Other soilborne pathogens considered among the most severe fungal diseases in the world are species of the genus *Verticillium*. Specifically, *Verticillium dahliae* Kleb. can infect over 200 plant species worldwide [32], causing the so-called *Verticillium* wilt, which damages developing orchards and reduces productivity by causing branch dieback in *Prunus* spp.

Concerning grapevine trunk diseases, *Botryosphaeriaceae* dieback is one of the most harmful, emerging, and widespread wood diseases currently affecting vineyards [33,34]. Species associated with the “dead arm” syndrome belong to the genera *Botryosphaeria, Neofusicoccum, Neoscytalidium, Phaeobotryosphaeria, Diplodia, Lasiodiplodia, Dothiorella, Spencermartinsia*, and *Sphaeropsis* [35]. Notably, among them is *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, a highly virulent and polyphagous pathogen that rapidly infects wood [36]. Due to climate change, it is becoming an emerging disease in *Rosaceae* plant species [37], increasing the need to understand its pathogenicity, particularly concerning economically valuable apple varieties [38]. *Diplodia seriata* is also noteworthy, causing acronecrosis, cankers, leaf spots, and fruit rot in a wide range of hosts, including grapevines [39] and apple trees [40].

As for bacterial diseases, *Erwinia amylovora* (Burrill, 1882) Winslow et al., 1920 and *Xylophilus ampelinus* (Panagopoulos, 1969) Willems et al., 1987 (syn. *Xanthomonas ampelina* Panagopoulos 1969 and *Erwinia vitivora* Du Plessis) are A2 quarantine organisms according to the European and Mediterranean Plant Protection Organization (EPPO). *E. amylovora*, the causal agent of fire blight —a devastating disease of grapes, apples, and pears— can be found in the updated review by Zhao *et al.* [41]. *X. ampelinus*, causing bacterial blight of grapevines (the "maladie d'Oléron" or "mal nero"), results in more than 70% crop losses [42], and its symptoms are often confused with those of "dead arm," caused by *Botryosphaeriaceae* fungi. As for *Pseudomonas syringae* pv. *syringae* van Hall, it causes bacterial spots on leaves and cankers and can affect species in the families *Fabaceae*, *Cruciferae*, *Solanaceae*, and *Rosaceae* [43].

Regarding grapevine aerial organs, powdery mildew (*Erysiphe necator* Schwein. synonym *Uncinula necator* (Schwein.) Burrill) is a particularly important disease [44]. Its incidence and severity are increasing as a consequence of climate change [45,46], and, in France, it has been estimated that its treatment accounts for about half of the production cost. Under favorable environmental conditions, the pressure of this disease forces the use of enormous quantities of phytosanitary products, which entails high economic and environmental costs and, in many cases, quickly generates resistance [47]. According to Eurostat data, the application of phytosanitary products per hectare per year in viticulture is the highest of all crops [48]. In some cases, the number of applications per growing season is higher than 12 [49], reaching up to 16 applications in times of high disease pressure. In a study on pecuniary and nonpecuniary costs of managing powdery mildew in California grape production, Sambucci *et al.* [50] estimated that powdery mildew control accounted for 89% of crop protection applications in this sector.

Among the pathogens that affect forest crops, *Fusarium circinatum* Nirenberg and O'Donnell is an invasive pathogen causing a disease known as pitch canker in pines. This fungus is a quarantine organism, listed in EPPO's (European and Mediterranean Plant Protection Organization) A2 category and regulated in the EU [51]. It is known in many pine-producing regions, including natural and planted forests, and can affect all stages of tree life, from emerging seedlings to mature trees. In nurseries and the general environment, the pathogen affects pines (*Pinus spp.*) and Douglas fir (*Pseudotsuga menziesii*). Therefore, it is currently one of the most significant threats to *Pinus spp.* worldwide [52].

Chestnut blight is a stem disease of *Castanea* caused by the pathogenic fungus *Cryphonectria parasitica* (Murr.) Barr. Chestnut blight affects to a greater or lesser extent all species of *Castanea*. In American and European species, chestnut blight has caused a significant decline in wild populations and continues to negatively impact nut production in the European chestnut (*Castanea sativa* Mill.). However, there is limited information on the factors involved in the host-pathogen interaction between *C. parasitica* and its hosts *Castanea* [53]

On the other hand, *Phytophthora cinnamomi* Rands causes "root and crown rot" in a wide range of hosts, mainly belonging to the genera *Castanea*, *Eucalyptus*, *Fagus*, *Juglans*, *Quercus*, etc. This oomycete is a threat to global food security and to the health, function, and biodiversity of native ecosystems, such as the *dehesa* [54], exacerbated by climate change [55]. *Phytophthora cinnamomi* is a globally distributed pathogen that can infect thousands of species and is considered a key biotic driver of the decline of *Quercus* spp. forests in Spain [56]. It is also one of the world's most threatening invasive pathogens [57]. Other pathogens threatening the trees of the *dehesa* are fungi of the genus *Botryosphaeria*, including *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. De Not., *Diplodia corticola* Phillips, Alves & Luque, and *Dothiorella iberica* Phillips, Luque & Alves, causing cankers and branch dieback and have been associated with the decline of oaks and cork oaks, although *B. dothidea* has also been found in other species of the genus *Quercus* such as *Quercus robur* L. and *Quercus rubra* Michx. L. [58].

In the group of staple food crops, wheat and maize are particularly important for their contribution to food security [59]. However, cereal production is threatened by climate change and plant disease epidemics [60]. For example, Fusarium head blight (FHB) severely reduces the quality and quantity of cereal production, such as wheat, maize, and barley [61], with the added issue of mycotoxin production. Mycotoxins are toxic low-molecular-weight secondary metabolites with high thermal stability and bioaccumulation capacity, potentially harmful to human and animal health [62].

In the context of horticultural crops, global economic losses caused by bacterial diseases have been estimated at over one billion dollars annually worldwide [63]. Among them, *Pseudomonas syringae* van Hall [64] and *Pseudomonas cichorii* (Swingle 1925) Stapp, 1928 [65] infect a wide range of plant species, while *Xanthomonas vesicatoria* (Doidge) Vauterin et al. is the causal agent of bacterial leaf spot in tomatoes (*Solanum lycopersicum* L.) and peppers (*Capsicum annuum* L.) [66], and *Clavibacter michiganensis* subsp. *michiganensis* corrig. (Smith 1918) Davis et al. 1984 causes bacterial canker of tomatoes, also potentially affecting other solanaceous plants [67]. It is worth noting that the pathovars of *P. syringae* and *C. michiganensis* subsp. *michiganensis* are considered among the most relevant bacterial pathogens due to their scientific/economic importance [68]. Also noteworthy are bacteria responsible for soft rot and blackleg of potatoes (*Solanum tuberosum* L.), such as *Pectobacterium carotovorum* subsp. *carotovorum* (Jones 1901) Hauben et al. 1999; *Pectobacterium atrosepticum* (van Hall 1902) Gardan et al. 2003; *Pectobacterium parmentieri* Khayi et al. 2016; and *Dickeya chrysanthemi* (Burkholder et al. 1953) Samson et al. 2005, which cause a viscous, wet, black rot lesion that spreads through the stems from the rotten mother tuber, especially in humid conditions. It affects both seed and consumable potato production, with estimated global losses of €46 million per year for the European sector (with significant variability between years) [69].

Horticultural crops are also affected by fungal pathogens. *Fusarium* species, which are ubiquitous soil-borne pathogens and cause destructive vascular wilts, rots, and damping-off diseases, are among the most destructive pathogens affecting these crops [70]. *Fusarium oxysporum* Schltdl causes Fusarium wilt and root rot, destroying up to 70% of the borage (*Borago officinalis* L.) crop in 2019 in Aragon [71]. *Fusarium oxysporum* f. sp. *niveum* (E.F. Sm.) Snyder & H.N. Hansen causes wilt in watermelons [72]. *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hansen causes vascular wilt of tomato disease, with a high impact on yield [73]. *Fusarium equiseti* (Corda) Sacc. not only affects the quality and quantity of cereal crops [61] but also causes crown and root rot in cucurbits [74].

The genus *Neocosmospora* (a taxon belonging to the so-called *Fusarium solani* species complex, FSSC) contains saprophytes, plant endophytes, and economically important pathogens [75]. *Neocosmospora falciformis* (Carrión) Summerb. & Schroers has been associated with the decline of various plant species, as well as with the wilting and root rot of muskmelon in Spain [76]. Another member of the complex is *Neocosmospora keratoplastica* Geiser, O'Donnell, Short & Zhang, which also affects cucurbits causing root rot and decay [77].

Macrophomina phaseolina (Tassi) Goid is a soil-borne pathogen that affects over 500 plant species, including melon, and is responsible for charcoal rot disease [78]. Symptoms include sunken, dark lesions at the base of the stem, as well as leaf and stem chlorosis, vine wilting, and stem and root rot [79].

Rhizoctonia solani Kühn has a broad host range of over 200 plant species, especially in the *Solanaceae* family, including eggplant, bell pepper, potato, tobacco, and cultivated tomatoes in both greenhouse and field conditions, causing the so-called root and foot rot [80]. In turn, *Alternaria alternata* (Fr.) Keissl. is responsible for different postharvest diseases of many horticultural products, causing black rot and black spot [81].

Another polyphagous pathogenic fungus, *Sclerotinia sclerotiorum* (Lib.) de Bary, causes stem rot or white mold in many commercially important crops, including cucurbits, leading to significant economic losses. Additionally, sclerotia production enables it to survive in infected tissues, crop residues, or soil for up to eight years [82].

In terms of postharvest fruit protection, *Botrytis cinerea* Pers. and *Colletotrichum* spp. rank second and eighth, respectively, on a list of scientifically and economically important pathogenic fungi [83]. *Botrytis cinerea* has a wide range of hosts (over 200 plant species) and can cause serious damage before and after harvest. It produces gray mold on the fruit and senescent organs but also affects vegetative tissues [84]. On the other hand, the fungal genus *Colletotrichum* includes numerous important phytopathogenic species and species complexes that infect a wide variety of hosts, causing postharvest anthracnose [85]. *Colletotrichum* species are widely distributed in tropical and subtropical regions [86]. Several species affect temperate and Mediterranean crops of high value such as strawberries (*Colletotrichum nymphaeae* (Pass.) Aa.), apples (*Colletotrichum acutatum* J.H.Simmonds), and various fruits and vegetables (*Colletotrichum coccodes* (Wallr.) Hughes) [87]

1.3. Objectives of the Doctoral Thesis

1.3.1. *Working hypothesis*

This Doctoral Thesis has been designed to tackle the challenge of mitigating the excessive use of chemical phytosanitary treatments in agriculture, aligning with the stipulations of the European Directive 2009/128/EC (Article 14) and its transposition into the Spanish Royal Decree 1311/2012. This initiative is particularly pertinent in a scenario where the prevalence of diseases of microbial origin in agroforestry crops is on the rise due to the impacts of climate change. The foundational hypothesis driving this research posits that conventional synthetic pesticides can potentially be substituted with natural products exhibiting equivalent antimicrobial efficacy.

1.3.2. *General objective*

The overarching goal of this Doctoral Thesis is to validate the effectiveness of treatments employing alternative products derived from natural sources in combating microbial diseases within agroforestry systems. This encompasses the assessment of plant extracts, extracts from medicinal mushrooms, and biocontrol agents (*Streptomyces* spp.).

1.3.3. *Specific objectives*

The general objective has been addressed through the following specific objectives:

- *Specific objective 1 (SO1)*: Synthesis and characterization of novel antimicrobial formulations based on naturally occurring bioactive species with improved extraction, solubility, and bioavailability.
- *Specific objective 2 (SO2)*: Conduct laboratory-scale tests, both *in vitro*, *in vivo*, or *ex-situ*, to validate the proposed optimization of treatments. This involves exploring synergies among different agents with antimicrobial activity.
- *Specific objective 3 (SO3)*: *In planta* scale tests of the optimized antimicrobial compositions on different crops with great relevance in agricultural and forestry production in the Iberian Peninsula.
- *Specific objective 4 (SO4)*: Technology transfer: preparation and processing of patents on the results.
- *Specific objective 5 (SO5)*: Dissemination of results to the scientific community, in indexed journals and national and international conferences.

1.4. Justification of the thematic unity of the articles

The Doctoral Thesis is composed of 19 scientific articles and three patents, which are detailed in the *"Report on the publications included in the Doctoral Thesis"* section of this document. This scientific production shares the evaluation of the efficacy of hydromethanolic and/or aqueous ammonia extracts (depending on the plant part under study) derived from natural sources. The focus is on controlling emerging fungal and bacterial diseases in the Iberian Peninsula. The research is organized into four main thematic blocks: woody crops, extensive (cereal) crops, horticultural crops, and post-harvest fruit protection.

Articles 1-7 are dedicated to the woody crops theme, focusing on evaluations against fungal pathogens in grapevines (*Erysiphe necator*, *D. seriata*, *D. viticola*, and *N. parvum*), apple trees (*M. fructigena*, *M. laxa*, and *P. cactorum*), and almond trees (*D. amygdali*, *P. megasperma*, and *V. dahliae*). Additionally, in articles 1, 2, 4, and 6 the broad-spectrum activity of natural products against phytopathogenic bacteria was also explored, including *E. amylovora*, *X. ampelinus*, and *P. syringae* pv. *syringae*. Regarding forest species, articles 8 and 9 address the management of emerging pathogens such as *F. circinatum*, *P. cinnamomi*, *B. dothidea*, *D. corticola*, and *D. iberica*, with *ex-situ* trials on pieces of wood to evaluate the protection of extracts against the oomycete *P. cinnamomi*.

Article 10 is the only representative of the herbaceous crops (cereals) theme, with *in vitro* tests on *Fusarium* spp., as well as on wheat and maize grain storage protection.

The horticultural crops block is made up of articles 11-13, where the effectiveness of extracts was assessed against bacterial pathogens, viz. *P. carotovorum* subsp. *carotovorum*, *P. atrosepticum*, *P. parmentieri*, *D. chrysanthemi*, *C. michiganensis* subsp. *michiganensis*, *P. cichorii*, *P. syringae* pv. *pisi*, *P. syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *X. vesicatoria* pv. *vesicatoria*, and —to a lesser extent — against pathogenic fungi such as *B. cinerea*, *F. oxysporum*, and *S. sclerotiorum*. Specifically, article 11 includes *ex-situ* tests on potato tubers, while articles 12 and 13 include *in planta* greenhouse-scale tests on pea and tomato plants, and borage, respectively.

Finally, the post-harvest protection block is made up of articles 14-19, with trials on post-harvest protection of fruits, specifically testing their efficacy against *B. cinerea* and *C. nymphaeae* in strawberries and grapes, *C. acutatum* in apples, *S. sclerotiorum* in cucumbers, *C. coccodes* in tomatoes.

1.5. General methodology

In this Doctoral Thesis, the methodology employed in the published articles on achieving the programmed objectives has been as follows:

Concerning *specific objective 1*, all publications worked with plant extracts in hydromethanolic or aqueous ammonia media, depending on the part of the plant under study. To improve solubility and bioavailability, they were combined with chitosan oligomers (COS) (articles 1, 2, 4-6, 9, 13, 14, and 16-18), using Green Chemistry techniques such as ultrasonication for the formation of conjugated complexes. All articles share the procedure for characterizing the functional groups present in the plant organs and extracts, which is based on the use of attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy, and the elucidation of extract phytoconstituents by gas chromatography/mass spectrometry (GC-MS). Additionally, in articles 1, 2, and 4, multi-elemental (CHNS) analyses were conducted.

Regarding *specific objective 2*, the methodology for determining *in vitro* antifungal activity was the same in all articles (EUCAST EDef 7.2 method [88]), and the determination of *in vitro* antibacterial activity in articles working with bacterial pathogens employed the CLSI M07–11 methodology

[89]. Concerning the methodology for *ex-situ* wood protection tests (articles 6-9), it was conducted on wood pieces susceptible to *Phytophthora* spp., following the protocol set by Matheron and Mircetich [90] and Álvarez Bernaola [91]. Tests for the protection of corn and wheat grains (article 10) were carried out following the protocol of Perczak *et al.* [92]. On the other hand, protection tests on potato slices (article 11) followed the methodology proposed by Abd-El-Khair *et al.* [93]. Regarding tests on post-harvest fruit protection, tests conducted on strawberries (articles 14, 16, and 18) followed the procedures defined by Hernández-Muñoz *et al.* [94] and Romanazzi *et al.* [95], and tests on grapes (article 16) followed the protocol proposed by Riquelme *et al.* [96]. In the case of apples (article 15), it was the one proposed by Pereira *et al.* [97] and Loebler *et al.* [98]. For cucumber protection (article 17), the protocol proposed by Onaran and Yanar [99], with slight modifications proposed in [100], was adopted. Finally, in tomato tests (article 19), the protocol proposed by Wang *et al.* [101] was followed.

Regarding specific *objective 3*, the methodology for *in-planta* tests on tomato and pea plants (article 12) and on borage plants (article 13) followed the methodology outlined by de León *et al.* [102], Martín Sanz *et al.* [103], and González *et al.* [71], respectively.

Concerning *specific objective 4*, the investigations collected in articles 27, 29, and 31, which are not part of the compilation of publications of this Doctoral Thesis (given that they are part of another Doctoral Thesis) but make use of the extracts explored herein, have been the subject of Spanish patent applications (one of which has been internationally extended). In all cases, funding was provided by the General Foundation of the University of Valladolid, thanks to the PROMETEO 2021, 2022, and 2023 awards, and with the support of ClarkeModet España and UNGRIA Patentes y Marcas for drafting and processing the patents.

In relation to *specific objective 5*, related to the dissemination of results to the scientific community, it has been addressed through publication in international JCR-indexed journals; through presentations at congresses and events organized by the Spanish Society of AgroEngineering, the Spanish Society of Horticultural Sciences, the Spanish Society of Phytopathology, and the University of Salamanca); in Spanish language journals ("Valorización de los fitoquímicos de la corteza de *Quercus ilex* L. subsp. *ballota* de las dehesas zamoranas de los Arribes del Duero", Anuario IEZ "Florián de Ocampo", 2021, 71-84; https://iezfloriandeocampo.com/anuarios/2021/); and resorting to non-indexed publications in scientific dissemination journals (see "La revolución de la nanotecnología en agricultura". *The Conversation*. Asociación The Conversation España, 11/05/2023, available at https://theconversation.com/la-revolucion-de-la-nanotecnologia-en-agricultura-191104, and the interview for the Biologicals Latam magazine entitled "Extractos vegetales para el control de plagas y enfermedades: El renacer de las plantas para la protección de los cultivos", available at https://biologicalslatam.com/ed/04/).

2. COMPENDIUM OF PUBLICATIONS

ARTICLE 1: "Characterization and antimicrobial activity of a halophyte from the Asturian coast (Spain): Limonium binervosum (G.E.Sm.) C.E.Salmon". Plants, 2021, 10(9), 1852, https://doi.org/10.3390/plants10091852, Q1 JCR (Science Edition - Plant Sciences, 39/238). JIF2021 = 4.658.

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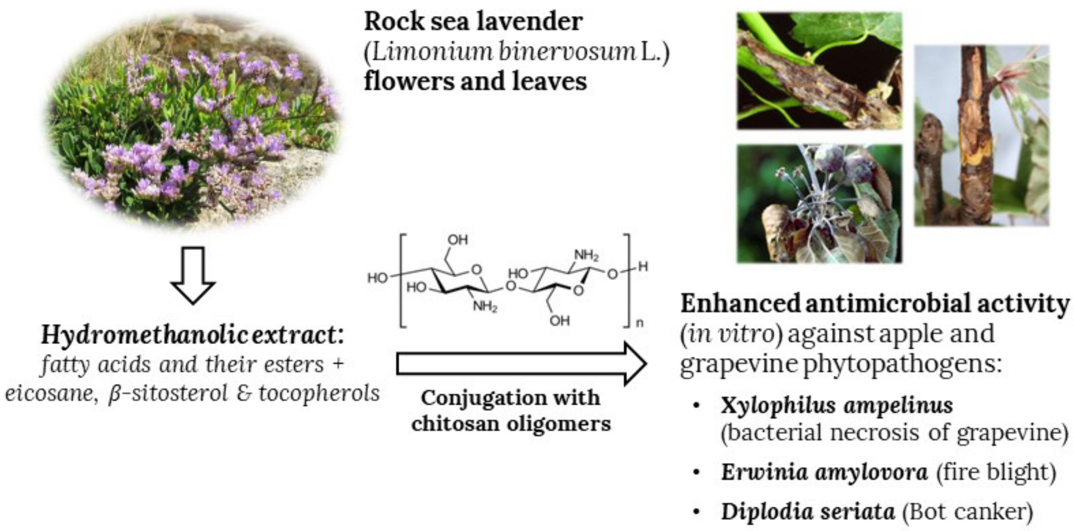
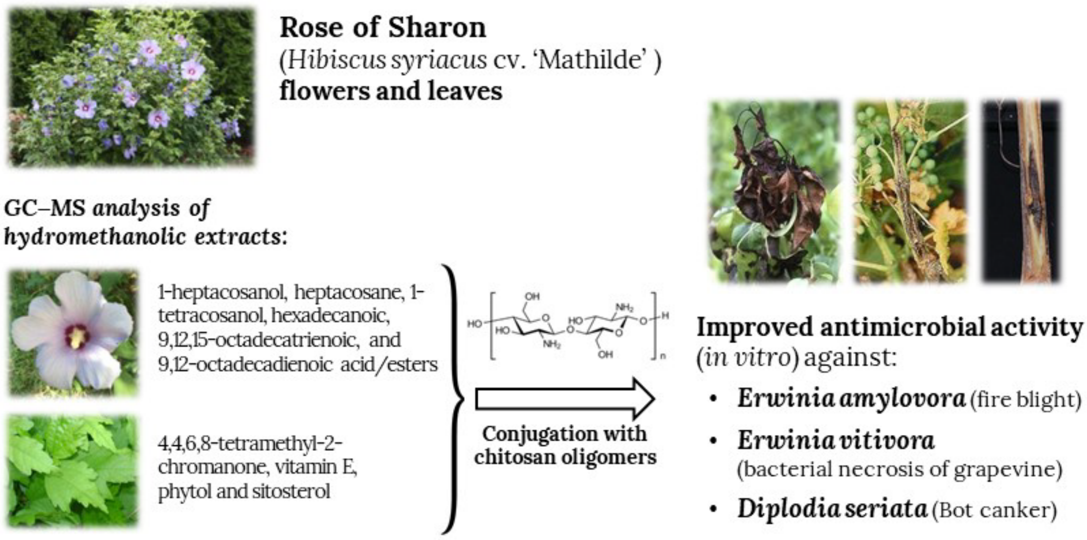
Characterization and Antimicrobial Activity of a Halophyte from the Asturian Coast (Spain): *Limonium binervosum* (G.E.Sm.) C.E.Salmon

Eva Sánchez-Hernández; Laura Buzón-Durán; Natalia Langa-Lomba; José Casanova-Gascón; Belén Lorenzo-Vidal; Jesús Martín-Gil; Pablo Martín-Ramos

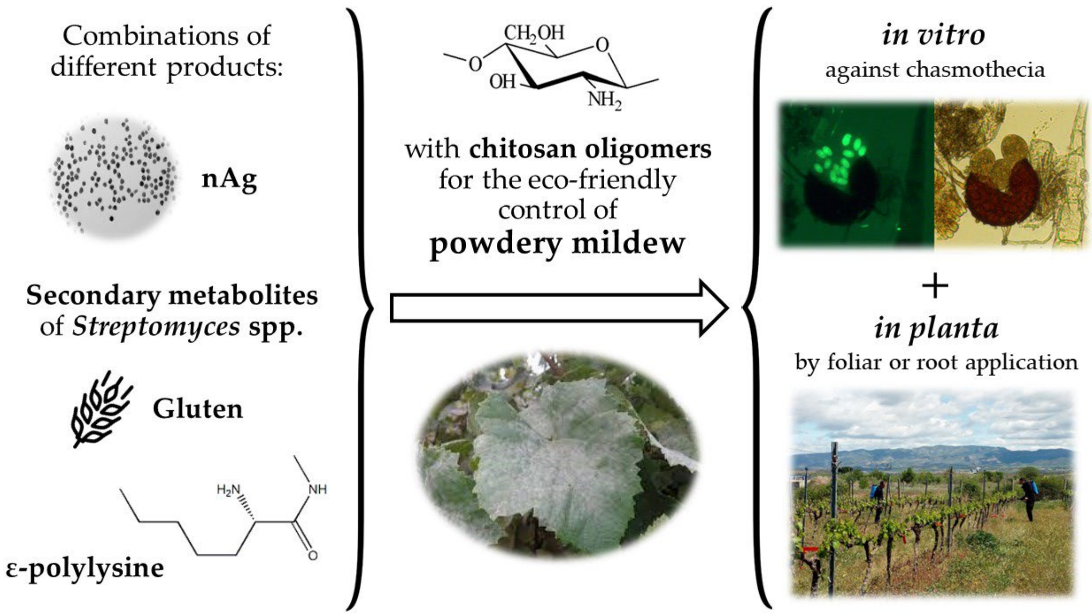
Plants 2021, Volume 10, Issue 9, 1852



ARTICLE 2: "Physicochemical characterization and antimicrobial activity against *Erwinia amylovora*, *Erwinia vitivora*, and *Diplodia seriata* of a light purple *Hibiscus syriacus* L. cultivar". *Plants*, 2021, 10(9), 1876, https://doi.org/10.3390/plants10091876; Q1 JCR (Science Edition - Plant Sciences, 39/238). JIF_{2021} = 4.658.



ARTICLE 3: "Chitosan-based bioactive formulations for the control of powdery mildew in viticulture". *Agronomy*, 2022, 12(2), 495, https://doi.org/10.3390/agronomy12020495; Q1 JCR (Science Edition – Agronomy, 16/88). JIF_{2022} = 3.7.



ARTICLE 4: "Dwarf pomegranate (*Punica granatum* L. var. *nana*): Source of 5-HMF and bioactive compounds with applications in the protection of woody crops". *Plants*, 2022, 11(4), 550, https://doi.org/10.3390/plants11040550; Q1 JCR (Science Edition - Plant Sciences, 43/238). $JIF_{2022} = 4.3$.

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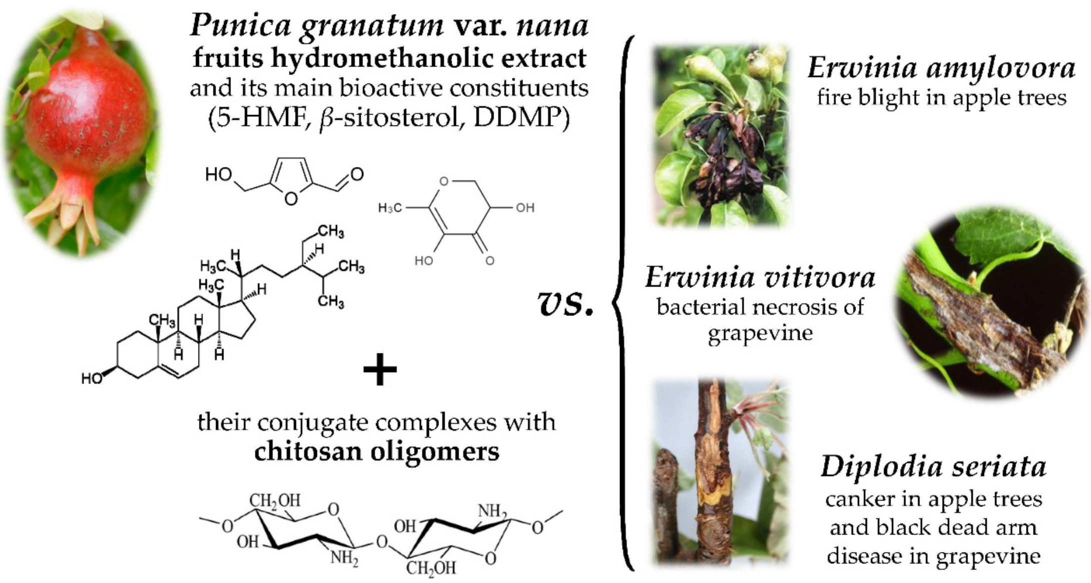
Dwarf Pomegranate (*Punica granatum* L. var. *nana*): Source of 5-HMF and Bioactive Compounds with Applications in the Protection of Woody Crops

Eva Sánchez-Hernández; Laura Buzón-Durán; José A. Cuchí-Oterino; Jesús Martín-Gil; Belén Lorenzo-Vidal; Pablo Martín-Ramos

Plants 2022, Volume 11, Issue 4, 550



ARTICLE 5: "Antifungal activity of methylxanthines against grapevine trunk diseases". *Agronomy*, 2022, 12(4), 885, https://doi.org/10.3390/agronomy12040885; Q1 JCR (Science Edition – Agronomy, 16/88). JIF_{2022} = 3.7.

ARTICLE 6: "Valorization of *Quercus suber* L. bark as a source of phytochemicals with antimicrobial activity against apple tree diseases". *Plants*, 2022, 11(24), 3415, https://doi.org/10.3390/plants11243415; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF2022 = 4.5.

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Valorization of *Quercus suber* L. Bark as a Source of Phytochemicals with Antimicrobial Activity against Apple Tree Diseases

Eva Sánchez-Hernández; Vicente González-García; José Casanova-Gascón; Juan J. Barriuso-Vargas; Joaquín Balduque-Gil; Belén Lorenzo-Vidal; Jesús Martín-Gil; Pablo Martín-Ramos

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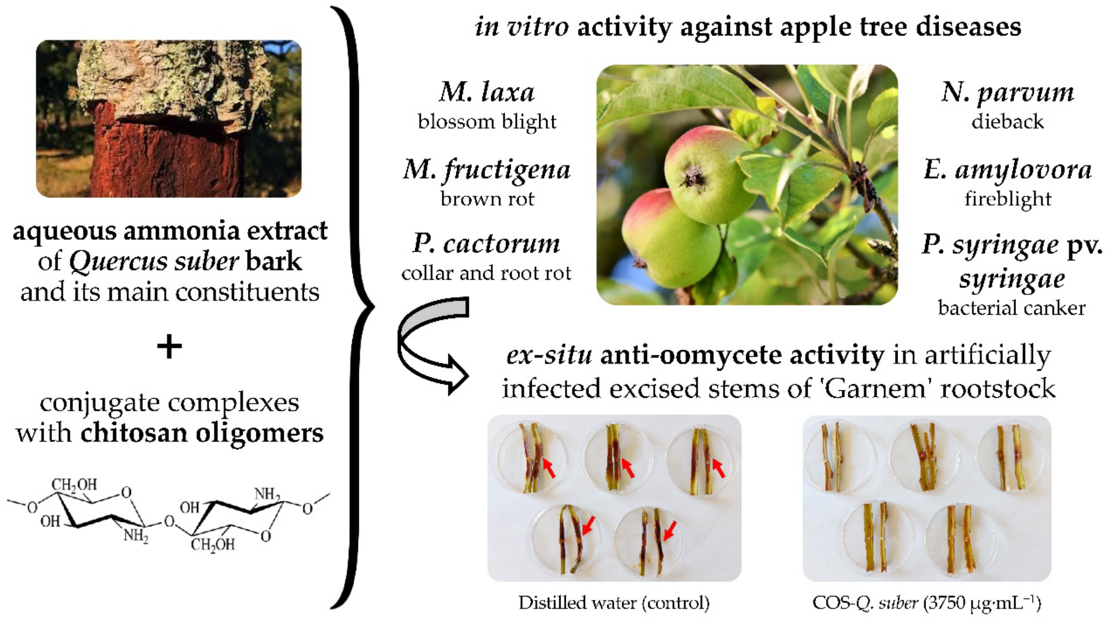
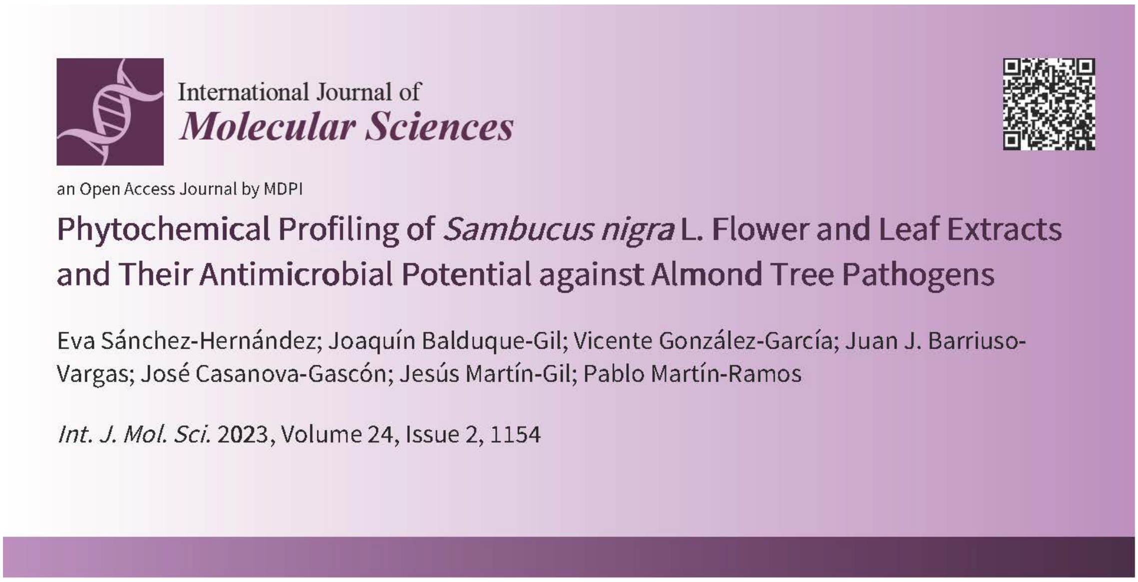
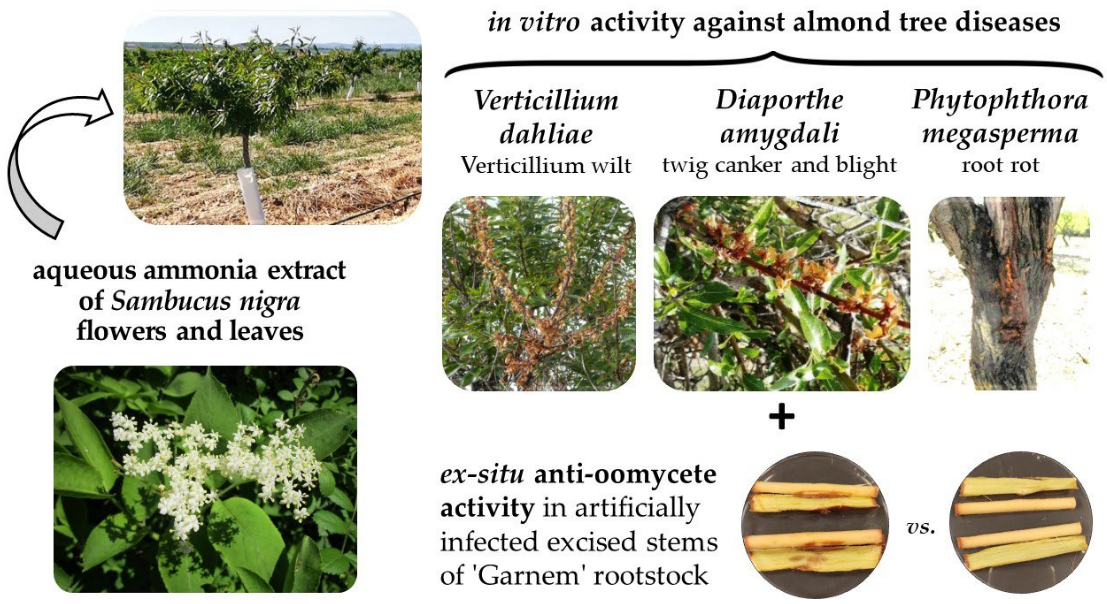
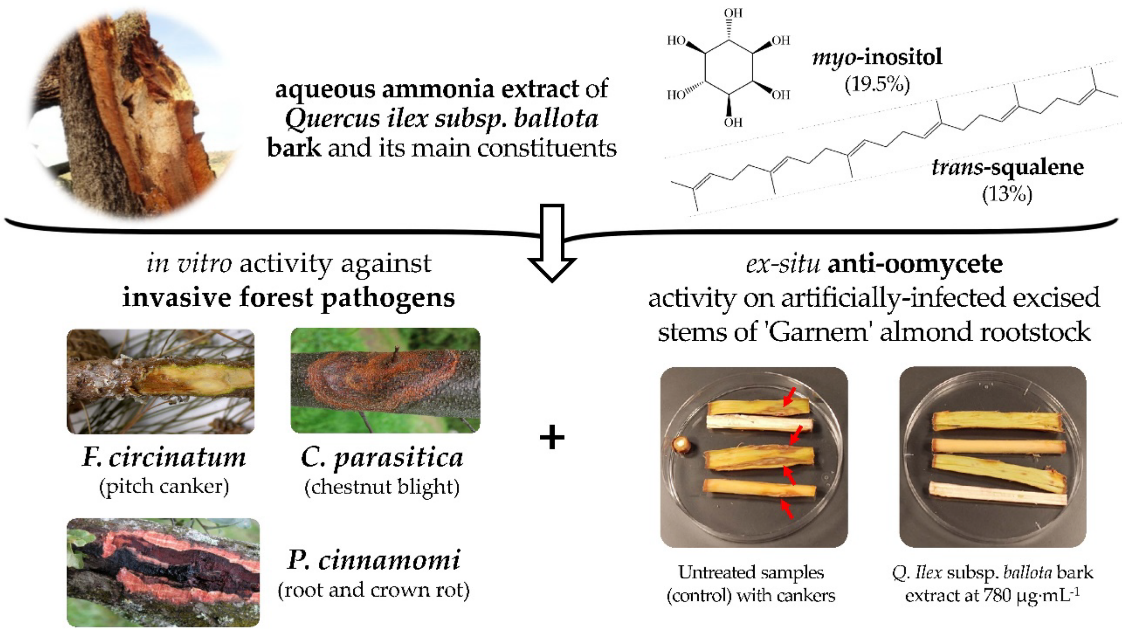


ARTICLE 7: "Phytochemical profiling of *Sambucus nigra* L. flower and leaf extracts and their antimicrobial potential against almond tree pathogens". *International Journal of Molecular Sciences*, 2023, 24(2), 1154, https://doi.org/10.3390/ijms24021154; Q1 JCR (Science Edition - Biochemistry & Molecular Biology, 66/285). JIF_{2022} = 5.6.





ARTICLE 8: "Holm oak (*Quercus ilex* subsp. *Ballota* (Desf.) Samp.) bark aqueous ammonia extract for the control of invasive forest pathogens". *International Journal of Molecular Sciences*, 2022, 23(19), 11882, https://doi.org/10.3390/ijms231911882; Q1 JCR (Science Edition - Biochemistry & Molecular Biology, 66/285). JIF2022 = 5.6.



ARTICLE 9: "Chemical constituents and antimicrobial activity of a *Ganoderma lucidum* (Curtis.) P. Karst. aqueous ammonia extract". *Plants*, 2023, 12(12), 2271, https://doi.org/10.3390/plants12122271; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF2022 = 4.5.

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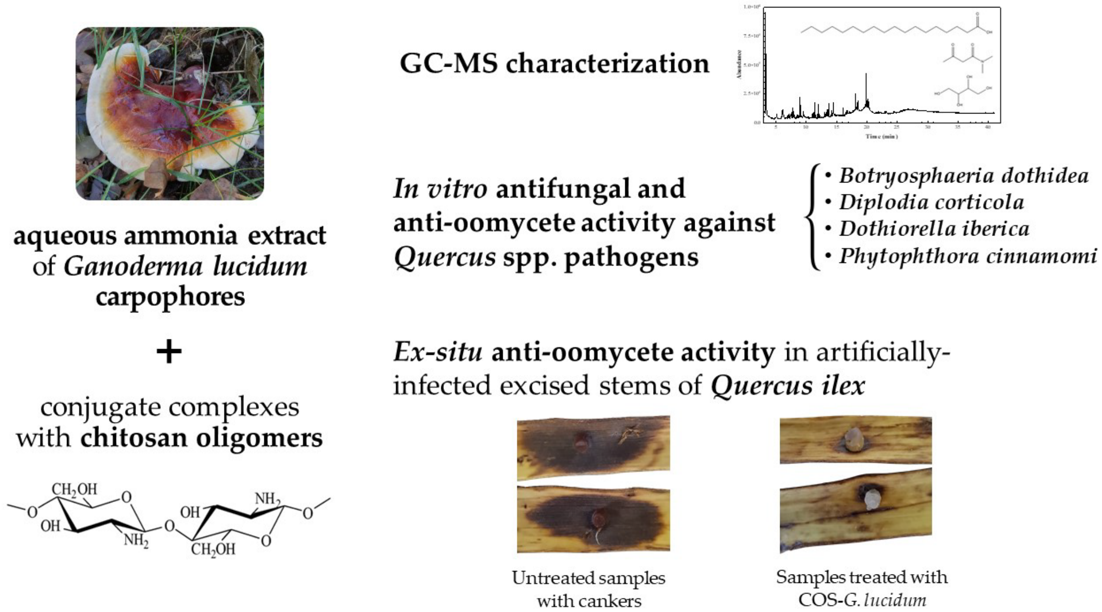
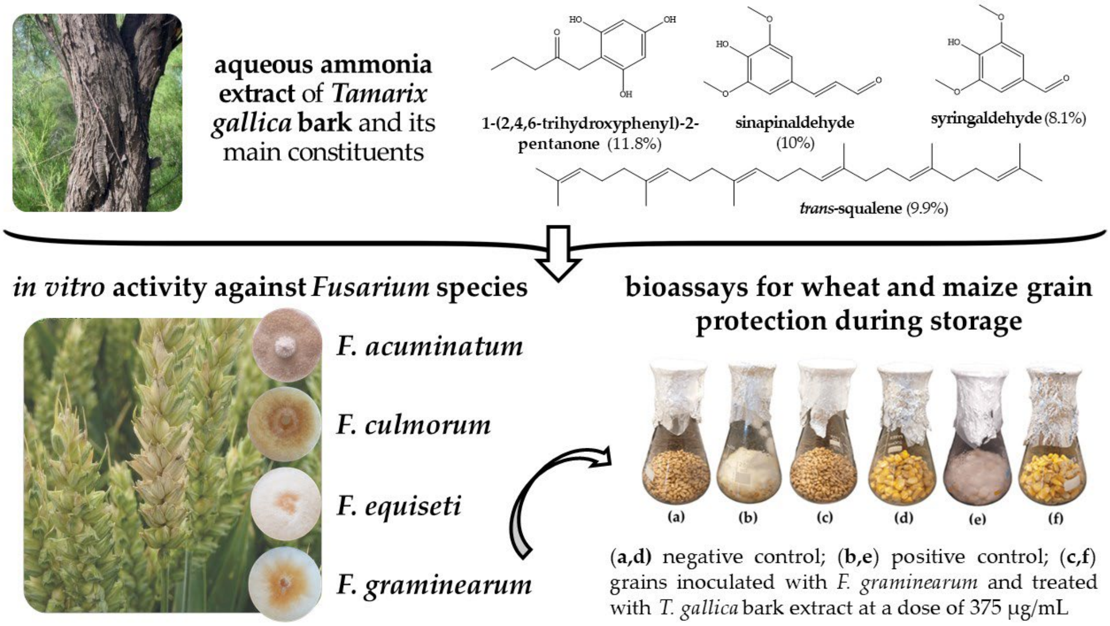
Chemical Constituents and Antimicrobial Activity of a *Ganoderma lucidum* (Curtis.) P. Karst. Aqueous Ammonia Extract

Eva Sánchez-Hernández; Ana Teixeira; Catarina Pereira; Adriana Cruz; Jesús Martín-Gil; Rui Oliveira; Pablo Martín-Ramos

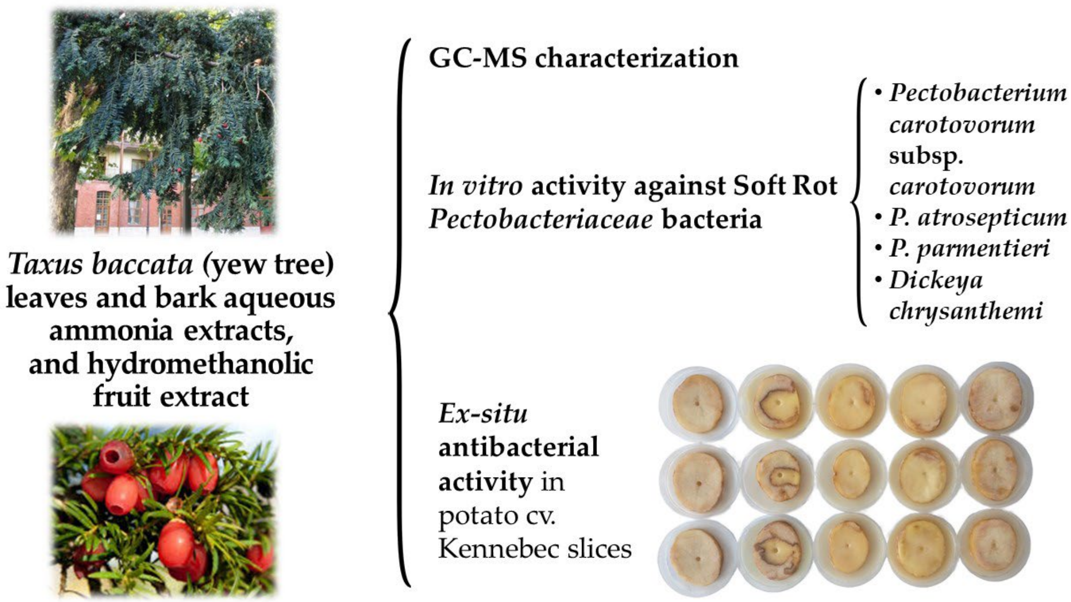
Plants 2023, Volume 12, Issue 12, 2271



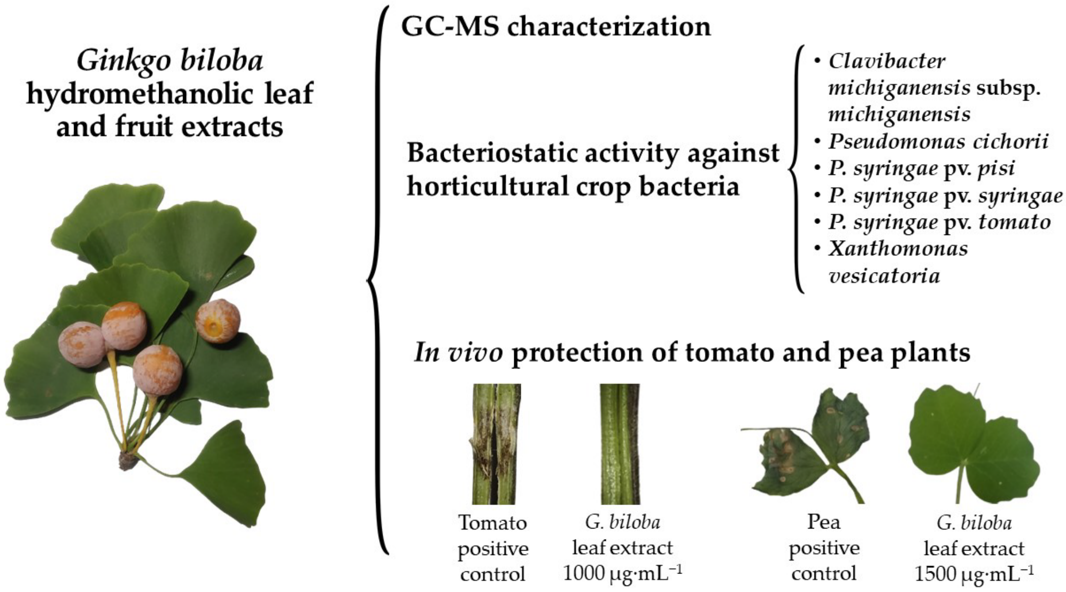
ARTICLE 10: "Phytochemical profile and activity against *Fusarium* species of *Tamarix gallica* bark aqueous ammonia extract". *Agronomy*, 2023, 13(2), 496, https://doi.org/10.3390/agronomy13020496; Q1 JCR (Science Edition – Agronomy, 16/88). JIF_{2022} = 3.7.



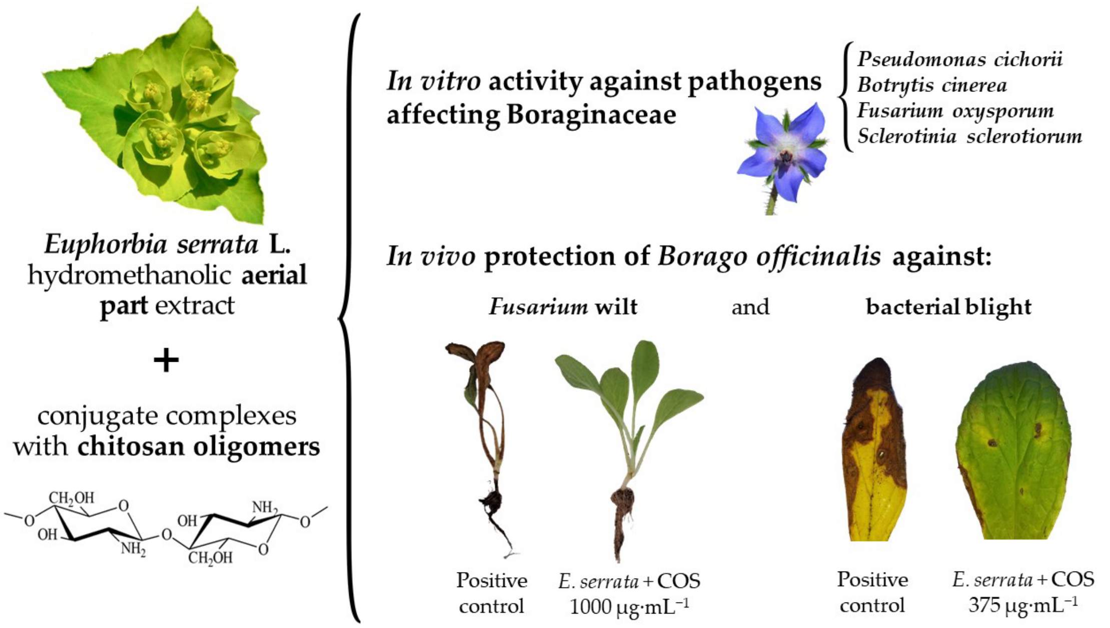
ARTICLE 11: "Phytochemical screening and antibacterial activity of *Taxus baccata* L. against *Pectobacterium* spp. and *Dickeya chrysanthemi*". *Horticulturae*, 2023, 9(2), 201, https://doi.org/10.3390/horticulturae9020201; Q1 JCR (Science Edition – Horticulture, 6/36). JIF2022 = 3.1.



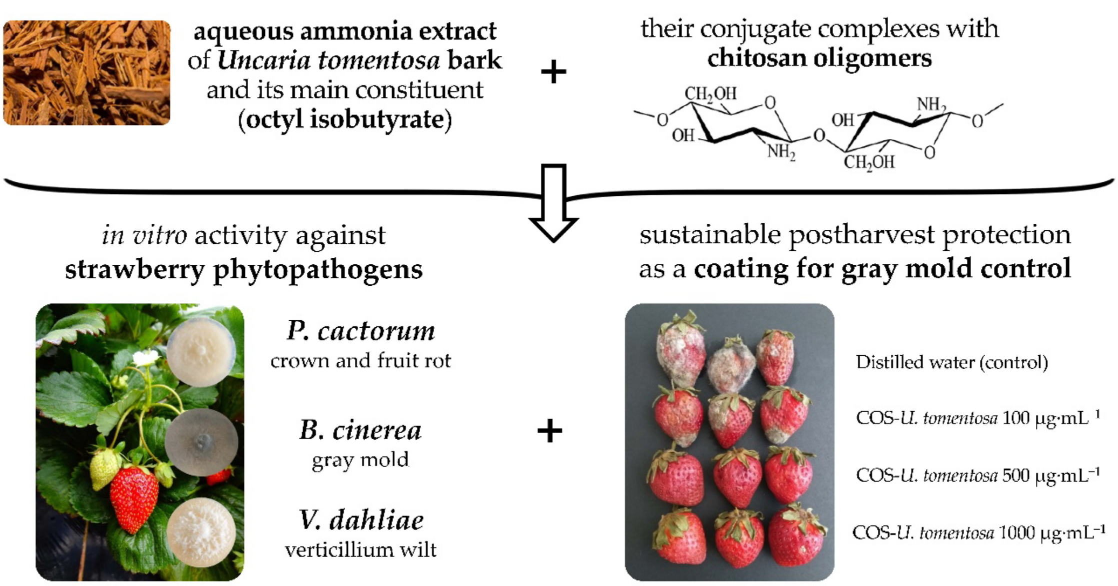
ARTICLE 12: "Antibacterial activity of *Ginkgo biloba* extracts against *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas* spp., and *Xanthomonas vesicatoria*". *Horticulturae*, 2023, 9(4), 461, https://doi.org/10.3390/horticulturae9040461; Q1 JCR (Science Edition – Horticulture, 6/36). JIF_{2022} = 3.1.



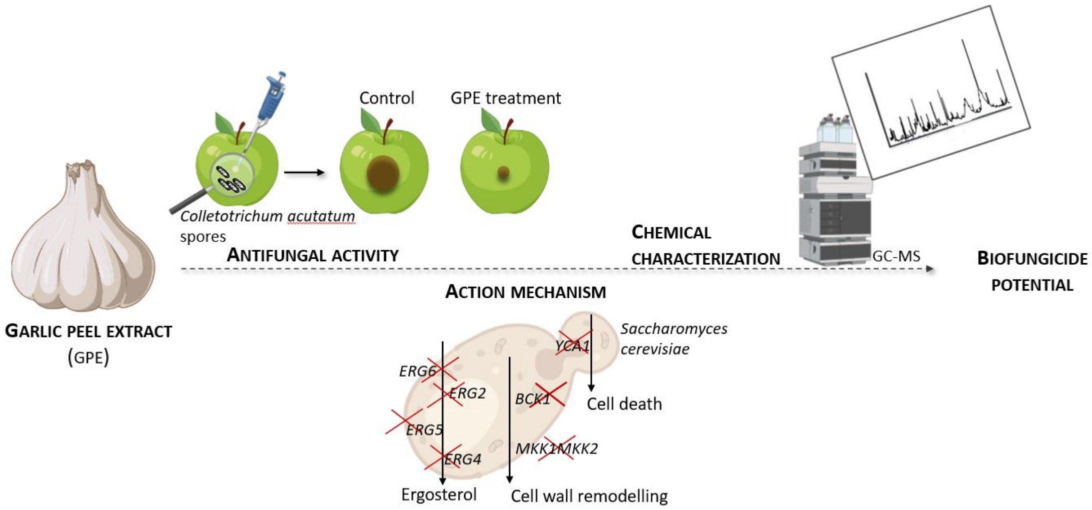
ARTICLE 13: "Phytochemical constituents and antimicrobial activity of *Euphorbia serrata* L. extracts for *Borago officinalis* L. crop protection". *Horticulturae*, 2023, 9(6), 652, https://doi.org/10.3390/horticulturae9060652; Q1 JCR (Science Edition – Horticulture, 6/36). JIF_{2022} = 3.1.



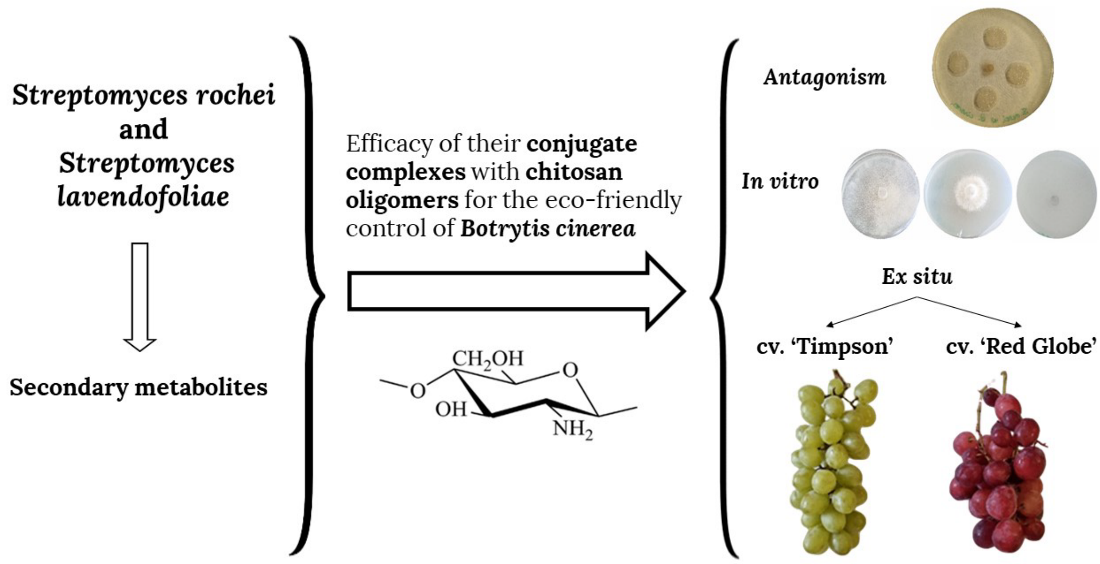
ARTICLE 14: "*Uncaria tomentosa* L. for the control of strawberry phytopathogens". *Horticulturae*, 2022, 8(8), 672, https://doi.org/10.3390/horticulturae8080672; Q1 JCR (Science Edition – Horticulture, 6/36). JIF_{2022} = 3.1.



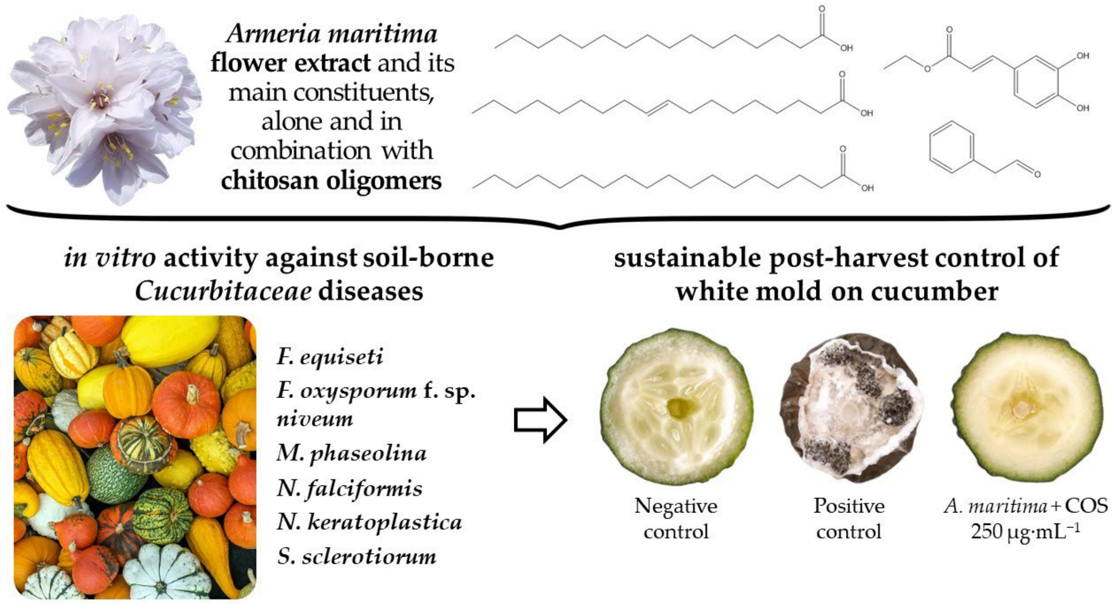
ARTICLE 15: "Antifungal activity of plant waste extracts against phytopathogenic fungi: *Allium sativum* peels extract as a promising product targeting the fungal plasma membrane and cell wall". *Horticulturae*, 2023, 9(2), 136, https://doi.org/10.3390/horticulturae9020136; Q1 JCR (Science Edition – Horticulture, 6/66). JIF_{2022} = 3.1.



ARTICLE 16: "A coating based on bioactive compounds from *Streptomyces* spp. and chitosan oligomers to control *Botrytis cinerea* preserves the quality and improves the shelf life of table grapes". *Plants*, 2023, 12(3), 577, https://doi.org/10.3390/plants12030577; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.5.



ARTICLE 17: "*Armeria maritima* (Mill.) Willd. flower hydromethanolic extract for Cucurbitaceae fungal diseases control". *Molecules*, 2023, 28(9), 3730, https://doi.org/10.3390/molecules28093730; Q2 JCR (Science Edition - Chemistry, Multidisciplinary, 63/178). JIF2022 = 4.6.



ARTICLE 18: "*Silene uniflora* extracts for strawberry postharvest protection". *Plants*, 2023, 12(9), 1846, https://doi.org/10.3390/plants12091846; Q1 JCR (Science Edition - Plant Sciences, 43/238). JIF_{2022} = 4.5.

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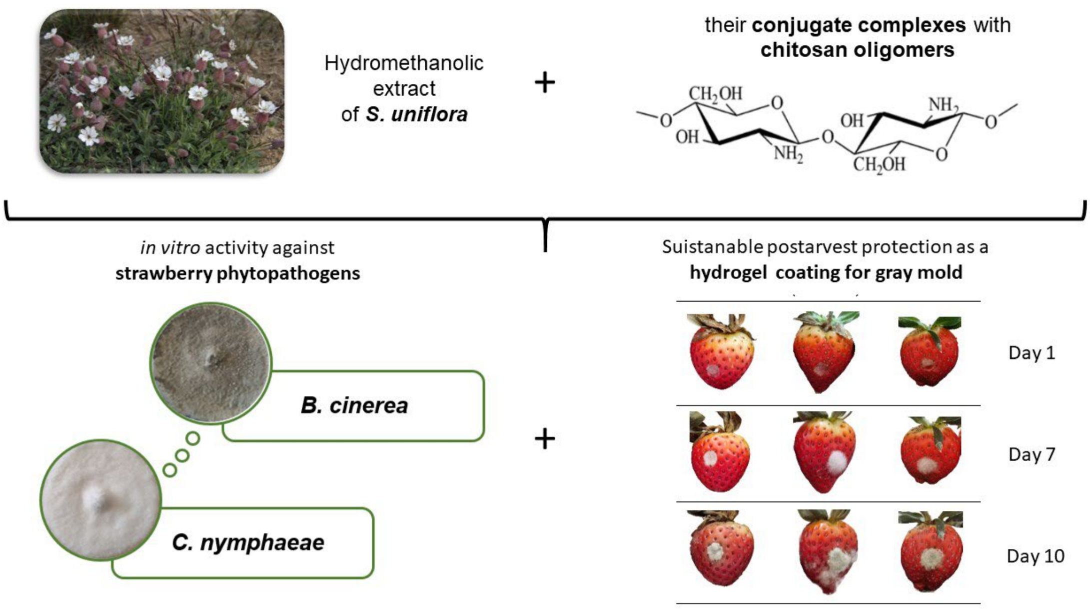
Silene uniflora Extracts for Strawberry Postharvest Protection

Laura Buzón-Durán; Eva Sánchez-Hernández; Pablo Martín-Ramos; Luis Manuel Navas-Gracia; Mari Cruz García-González; Rui Oliveira; Jesús Martín-Gil

Plants 2023, Volume 12, Issue 9, 1846



ARTICLE 19: "*Helichrysum stoechas* (L.) Moench inflorescence extract for tomato disease management". *Molecules*, 2023, 28(15), 5861, https://doi.org/10.3390/molecules28155861; Q2 JCR (Science Edition - Chemistry, Multidisciplinary, 63/178). JIF_{2022} = 4.6.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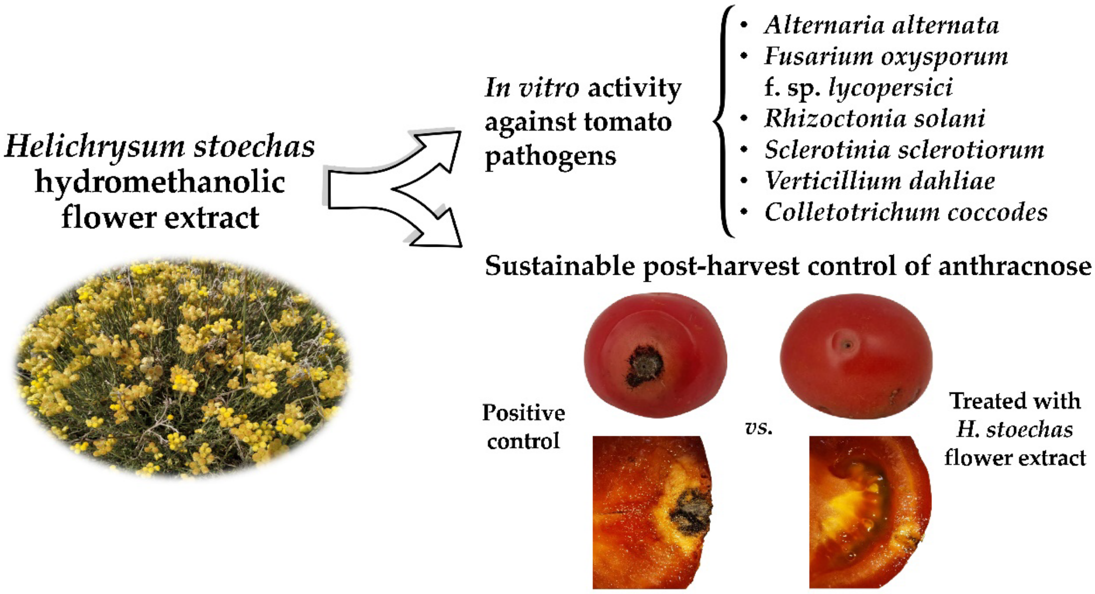
Helichrysum stoechas (L.) Moench Inflorescence Extract for Tomato Disease Management

Eva Sánchez-Hernández; Javier Álvarez-Martínez; Vicente González-García; José Casanova-Gascón; Jesús Martín-Gil; Pablo Martín-Ramos

Molecules 2023, Volume 28, Issue 15, 5861



3. DISCUSSION

In-depth analyses of the comparative effectiveness of each extract, concerning other natural products, are expounded in the corresponding sections of the individual manuscripts presented in the preceding section. Building upon these insights, this general discussion section aims to shift the focus towards a broader perspective, honing in on a comparative analysis between the investigated natural extracts and conventional fungicides. This intentional narrowing of the scope aligns with the initial hypothesis and overarching objective of the Thesis, allowing for an evaluation of the potential of natural products as alternatives to traditional synthetic fungicides.

In this Thesis, three types of conventional fungicides were utilized as benchmarks for efficacy comparisons: azoxystrobin (a strobilurin), mancozeb (a dithiocarbamate, currently prohibited for use), and fosetyl-Al (an organophosphorus compound).

Azoxystrobin, despite its broad-spectrum, rapid, and highly efficient germicidal activities, and cost-effectiveness, poses potential long-term adverse effects on the ecosystem. The rapid degradation of strobilurins during plant metabolism is advantageous; however, concerns have arisen regarding environmental contamination and non-target toxicity due to their prolonged use. Azoxystrobin has been frequently detected in foodstuffs, prompting serious public health concerns, as reported by the European Food Safety Authority (EFSA) [104]. Elevated environmental concentrations of azoxystrobin exceeding the regulatory acceptable concentration have been observed, posing a substantial risk to soil organisms, aquatic organisms, and mammals [105]. Furthermore, the susceptibility of strobilurins to resistance, attributed to their action on one specific site of fungal pathogens, has been documented, with multiple resistance genes reported in strobilurin-treated fungi [106].

In turn, dithiocarbamate pesticides, a significant class of organic fungicides globally used to control various diseases in crops (particularly certain ethylenebisdithiocarbamates, like mancozeb) have demonstrated properties typical of human carcinogens [107]. In response to this, the European Commission banned the use of mancozeb in January 2021 [108].

The third reference product, a phosphonic acid-based fungicide (or phosphonate), is widely employed as a crop protectant in horticulture. Phosphonates have played a crucial role in safeguarding sensitive forests and natural ecosystems threatened by pathogens. Since their introduction, they have showcased versatility in agriculture and beyond [109]. Although phosphonic acid and its salts and esters do not qualify as environmental contaminants, excessive doses applied to crops could yield toxic outcomes for plants [110]. On a positive note, in soil, phosphonates have been demonstrated to have no adverse impact on bacterial communities [111], and when applied as a foliar spray, they exhibit harmlessness against biological control agents [112].

Table 1, Table 2, and Table 3 present the results from selected articles in each thematic area (woody crops, cereal crops, and horticultural crops, respectively). It is worth noting that no comparisons are provided for the articles in the post-harvest protection theme, as current Spanish national legislation on the registration of phytosanitary products generally does not authorize fungicides for post-harvest protection.

In the context of woody crops, the *Q. suber* extract-COS conjugate complex demonstrated notable superiority over azoxystrobin and fosetyl-Al commercial fungicides when combating *Monilinia* spp., *N. parvum*, and *P. cactorum*. However, it fell short of reaching the efficacy level of mancozeb. Conversely, the *S. nigra* extract exhibited comparable efficacy to mancozeb against *V. dahliae* and superior activity against *D. amygdali* and *P. megasperma*. Against *P. cinnamomi*, extracts from *Q. ilex* subsp. *ballota* and *G. lucidum*-COS conjugate complex displayed significantly higher efficacy compared to the two conventional fungicides tested (azoxystrobin and fosetyl-Al). The former extract also yielded minimum inhibitory concentration (MIC) values over three orders of magnitude lower than azoxystrobin against *F. circinatum* and *C. parasitica*, while the latter outperformed fosetyl-Al against *Botryosphaeriaceae* fungi (refer to Table 1).

In the domain of cereal crops, the *T. gallica* extract demonstrated greater effectiveness against *Fusarium* spp. than both fosetyl-Al and mancozeb (see Table 2).

Concerning horticultural crops, the results obtained with the *E. serrata*-COS conjugate complex (refer to Table 3) surpassed those achieved with azoxystrobin and fosetyl-Al, albeit without reaching the efficacy level of mancozeb.

In light of these findings, it can be asserted that the initial hypothesis appears to be largely validated. The anticipation of resistance development is not foreseen, given that the plant extracts consist of mixtures of various phytochemicals exhibiting diverse modes of antimicrobial action. However, a note of caution is warranted, and it is imperative to underscore that the confirmation of these observations necessitates long-term field tests of the natural products.

Table 1. Comparison of MIC values for conventional fungicides and natural products against woody crop pathogens.

Pathogen	Commercial fungicide			Natural product			
	Azoxystrobin	Mancozeb	Fosetyl-Al	*Q. suber*-COS	*S. nigra* flower	*Q. ilex*	*G. lucidum*-COS
M. fructigena	>625,000	150	20,000	1000			
M. laxa	>625,000	150	20,000	750			
N. parvum	>625,000	150	20,000	750			
P. cactorum	62,500	150	2000	375			
D. amygdali	625,000	1500	20,000		1000		
P. megasperma	>625,000	15,000	20,000		375		
V. dahliae	625,000	1500	2000		1500		
F. circinatum	>625,000					375	
C. parasitica	>625,000					375	
P. cinnamomi	>625,000		2000			78.12	78.12
B. dothidea			2000				500
D. corticola			2000				500
D. iberica			2000				375

Table 2. Comparison of MIC values for conventional fungicides and natural products against cereal crop pathogens.

Pathogen	Commercial fungicide		Natural product
	Mancozeb	Fosetyl-Al	*T. gallica*
F. acuminatum	1500	20,000	1000
F. culmorum	15,000	2000	1000
F. equiseti	15,000	>20,000	750
F. graminearum	15,000	2000	375

Table 3. Comparison of MIC values for conventional fungicides and natural products against horticultural crop pathogens.

Pathogen	Commercial fungicide			Natural product
	Azoxystrobin	Mancozeb	Fosetyl-Al	*E. serrata*-COS
B. cinerea	>62,500	150	2000	750
F. oxysporum	>62,500	150	2000	500
S. sclerotiorum	>62,500	150	>2000	500

4. CONCLUSIONS

The primary conclusions derived from the research presented in this Thesis are as follows:

1. Protection of woody crops: extracts from *Q. suber*, *S. nigra*, and *G. lucidum* have proven noteworthy in the safeguarding of woody crops. Their demonstrated efficacies either surpass or are comparable to those of synthetic fungicides. Among them, the aqueous ammonia extract from *Quercus ilex* subsp. *ballota* emerges as the preferred fungicide/oomyceticide, with MICs below 400 ppm against various pathogens. Moreover, it afforded the highest protection in *ex-situ* assays against *Phytophthora* spp. In terms of antibacterial activity, all tested extracts (*L. binervosum, H. syriacus*, *P. granatum* var. *nana*, and *Q. suber*) exhibit inhibition values below 1500 ppm, with the hydromethanolic extract from *H. syriacus* flowers proving most effective against the most relevant studied pathogen, *E. amylovora*.

2. Cereal crop protection: the aqueous ammonia bark extract from *T. gallica* holds promise for cereal crop protection, surpassing the fungicidal activity of synthetic fungicides, including the currently prohibited mancozeb. Its efficacy extends to both preharvest and postharvest stages, ensuring complete protection of wheat and maize grains against *Fusarium* spp. This extract promotes storability and food safety without compromising seed germination rates.

3. Horticultural crops: assayed plant extracts (*T. baccata, G. biloba*, and *E. serrata*) exhibit high protection against bacterial phytopathogens. *E. serrata*, in particular, proves versatile by confirming protection against fungal pathogens, establishing it as a multifaceted biorational solution.

4. Postharvest protection: extracts tested for postharvest protection (*U. tomentosa, A. sativum*, *A. maritima, S. uniflora*, and *H. stoechas*) demonstrate substantial efficacy in *ex-situ* assays against fungi, significantly prolonging the shelf-life of studied fruits. Conjugate complexes of COS with *U. tomentosa* and *A. maritima*, and *H. stoechas* extract emerge as attractive solutions for controlling *B. cinerea, S. sclerotiorum*, and *Colletotrichum* spp., respectively, in light of current restrictions on synthetic fungicides in postharvest storage.

5. Conjugate complexes with COS: the formation of conjugate complexes with COS leads to a notable improvement in treatment efficacies, resulting in minimum inhibitory concentrations below 1000 ppm across all tested extracts and pathogens. This enhancement is attributed not only to synergistic behavior, given COS's antimicrobial properties, but also to increased solubility and bioavailability of active principles in the extracts. Additionally, COS imparts viscosity to the formulations, enhancing applicability via spray coating or dipping.

6. Broad applicability and long-term efficacy: the utilization of plant extracts, whether in hydromethanolic or aqueous ammonia media (depending on the plant part), alone or as conjugated complexes with COS, proves comparable in efficacy to widely used synthetic pesticides for controlling both pre and post-harvest species in crops of national and international significance. Beyond the advantages of non-toxicity and environmental friendliness, these treatments are anticipated to offer longer-term efficacy. Their complex nature fosters synergies among phytocomponents, preventing resistance development and providing a broader spectrum of activity.

Future lines of research

The exploration of future research avenues stemming from the completion of this Doctoral Thesis encompasses the following considerations:

- The efficacy of bioactive natural products can be impeded by challenges related to stability and solubility. An innovative avenue for overcoming these limitations and maximizing the effectiveness of such products involves micro/nanoencapsulation. The synthesis of nanocarriers using natural polymers, such as chitosan or alginate, holds promise for efficiently delivering natural products and enabling their selective release through exposure to the secretome of phytopathogens. This approach, aligned with the APLICADRON project (where the PhD Candidate serves as the Principal Investigator) and the three patents presented in the compendium, has the potential to yield significant savings in active ingredients.
- Despite the promising potential of nanocarriers, their application introduces challenges that merit further exploration in future research. In the preharvest stage, optimization of application methods for encapsulated natural products is crucial. This entails assessing their suitability for diverse approaches such as endotherapy, root application, foliar application, or drone-based delivery, depending on the specific crop under investigation. Ensuring the innocuousness of these encapsulated products on the final product's quality is an additional imperative.
- The application of nanotechnology-based solutions extends to postharvest protection, where the exploration of active or innovative packaging becomes pivotal. Future research endeavors should delve into the development of packaging solutions that align with the requirements set forth by the European Food Safety Authority. This involves ensuring not only the efficacy of these packaging methods in preserving the quality of agricultural products but also their compliance with stringent safety standards.

REFERENCES

1. Mancosu, N.; Snyder, R.; Kyriakakis, G.; Spano, D. Water scarcity and future challenges for food production. *Water* **2015**, *7*, 975-992, doi:10.3390/w7030975.
2. Matrose, N.A.; Obikeze, K.; Belay, Z.A.; Caleb, O.J. Plant extracts and other natural compounds as alternatives for post-harvest management of fruit fungal pathogens: A review. *Food Biosci.* **2021**, *41*, 100840, doi:10.1016/j.fbio.2020.100840.
3. Savary, S.; Ficke, A.; Aubertot, J.-N.; Hollier, C. Crop losses due to diseases and their implications for global food production losses and food security. *Food Security* **2012**, *4*, 519-537, doi:10.1007/s12571-012-0200-5.
4. Mauser, W.; Klepper, G.; Zabel, F.; Delzeit, R.; Hank, T.; Putzenlechner, B.; Calzadilla, A. Global biomass production potentials exceed expected future demand without the need for cropland expansion. *Nat. Commun.* **2015**, *6*, 8946, doi:10.1038/ncomms9946.
5. Gonzalez-Andujar, J.L.; Pradhan, P.; Fischer, G.; van Velthuizen, H.; Reusser, D.E.; Kropp, J.P. Closing yield gaps: How sustainable can we be? *Plos One* **2015**, *10*, e0129487, doi:10.1371/journal.pone.0129487.
6. Valin, H.; Sands, R.D.; van der Mensbrugghe, D.; Nelson, G.C.; Ahammad, H.; Blanc, E.; Bodirsky, B.; Fujimori, S.; Hasegawa, T.; Havlik, P.; Heyhoe, E.; Kyle, P.; Mason-D'Croz, D.; Paltsev, S.; Rolinski, S.; Tabeau, A.; van Meijl, H.; von Lampe, M.; Willenbockel, D. The future of food demand: understanding differences in global economic models. *Agricultural Economics* **2014**, *45*, 51-67, doi:10.1111/agec.12089.
7. Collinge, D.B.; Jørgensen, H.J.L.; Latz, M.A.C.; Manzotti, A.; Ntana, F.; Rojas, E.C.; Jensen, B. Searching for Novel Fungal Biological Control Agents for Plant Disease Control Among Endophytes. In *Endophytes for a Growing World*, 2019; 10.1017/9781108607667.003pp. 25-51.
8. Lamichhane, J.R.; Dachbrodt-Saaydeh, S.; Kudsk, P.; Messéan, A. Toward a reduced reliance on conventional pesticides in European agriculture. *Plant Disease* **2016**, *100*, 10-24, doi:10.1094/pdis-05-15-0574-fe.
9. Raymaekers, K.; Ponet, L.; Holtappels, D.; Berckmans, B.; Cammue, B.P.A. Screening for novel biocontrol agents applicable in plant disease management – A review. *Biological Control* **2020**, *144*, 104240, doi:10.1016/j.biocontrol.2020.104240.
10. Kim, K.-H.; Kabir, E.; Jahan, S.A. Exposure to pesticides and the associated human health effects. *Sci. Total Environ.* **2017**, *575*, 525-535, doi:10.1016/j.scitotenv.2016.09.009.
11. Price, C.L.; Parker, J.E.; Warrilow, A.G.S.; Kelly, D.E.; Kelly, S.L. Azole fungicides - understanding resistance mechanisms in agricultural fungal pathogens. *Pest Management Science* **2015**, *71*, 1054-1058, doi:10.1002/ps.4029.
12. van den Bosch, F.; Lopez-Ruiz, F.; Oliver, R.; Paveley, N.; Helps, J.; van den Berg, F. Identifying when it is financially beneficial to increase or decrease fungicide dose as resistance develops. *Plant Pathology* **2017**, *67*, 549-560, doi:10.1111/ppa.12787.
13. Bruce, T.J.A.; Smart, L.E.; Birch, A.N.E.; Blok, V.C.; MacKenzie, K.; Guerrieri, E.; Cascone, P.; Luna, E.; Ton, J. Prospects for plant defence activators and biocontrol in IPM – Concepts and lessons learnt so far. *Crop Protection* **2017**, *97*, 128-134, doi:10.1016/j.cropro.2016.10.003.
14. Pertot, I.; Caffi, T.; Rossi, V.; Mugnai, L.; Hoffmann, C.; Grando, M.S.; Gary, C.; Lafond, D.; Duso, C.; Thiery, D.; Mazzoni, V.; Anfora, G. A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Protection* **2017**, *97*, 70-84, doi:10.1016/j.cropro.2016.11.025.
15. Rijal, J.; Regmi, R.; Ghimire, R.; Puri, K.; Gyawaly, S.; Poudel, S. Farmers' knowledge on pesticide safety and pest management practices: A case study of vegetable growers in Chitwan, Nepal. *Agriculture* **2018**, *8*, 16, doi:10.3390/agriculture8010016.
16. Chapman, P. Is the regulatory regime for the registration of plant protection products in the EU potentially compromising food security? *Food and Energy Security* **2014**, *3*, 1-6, doi:10.1002/fes3.45.
17. Damalas, C.A.; Koutroubas, S.D. Botanical pesticides for eco-friendly pest management. In *Pesticides in Crop Production*, Srivastava, P.K., Singh, V.P., Singh, A., Tripathi, D.K., Singh,

S., Prasad, S.M., Chauhan, D.K., Eds. John Wiley & Sons Ltd: Hoboken, NJ, 2020; 10.1002/9781119432241.ch10pp. 181-193.

18. Marrone, P.G. Pesticidal natural products – status and future potential. *Pest Management Science* **2019**, 10.1002/ps.5433, doi:10.1002/ps.5433.
19. Suteu, D.; Rusu, L.; Zaharia, C.; Badeanu, M.; Daraban, G. Challenge of utilization vegetal extracts as natural plant protection products. *Applied Sciences* **2020**, *10*, 8913, doi:10.3390/app10248913.
20. Chaudhary, S. Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. *Frontiers in Plant Science* **2017**, *8*, 610, doi:10.3389/fpls.2017.00610.
21. Nxumalo, K.A.; Aremu, A.O.; Fawole, O.A. Potentials of medicinal plant extracts as an alternative to synthetic chemicals in postharvest protection and preservation of horticultural crops: A review. *Sustainability* **2021**, *13*, 5897, doi:10.3390/su13115897.
22. Sharma, R.R.; Alemwati, P. Natural products for postharvest decay control in horticultural produce: A review. *Stewart Postharvest Review* **2010**, *6*, 1-9, doi:10.2212/spr.2010.4.1.
23. Alonso-Gato, M.; Astray, G.; Mejuto, J.C.; Simal-Gandara, J. Essential oils as antimicrobials in crop protection. *Antibiotics* **2021**, *10*, 34, doi:10.3390/antibiotics10010034.
24. Hintz, T.; Matthews, K.K.; Di, R. The use of plant antimicrobial compounds for food preservation. *BioMed Research International* **2015**, *2015*, 1-12, doi:10.1155/2015/246264.
25. Li, K.; Xing, R.; Liu, S.; Li, P. Chitin and chitosan fragments responsible for plant elicitor and growth stimulator. *J. Agric. Food. Chem.* **2020**, *68*, 12203-12211, doi:10.1021/acs.jafc.0c05316.
26. Ma, Z.; Garrido-Maestu, A.; Jeong, K.C. Application, mode of action, and in vivo activity of chitosan and its micro- and nanoparticles as antimicrobial agents: A review. *Carbohydr. Polym.* **2017**, *176*, 257-265, doi:10.1016/j.carbpol.2017.08.082.
27. Ristaino, J.B.; Anderson, P.K.; Bebber, D.P.; Brauman, K.A.; Cunniffe, N.J.; Fedoroff, N.V.; Finegold, C.; Garrett, K.A.; Gilligan, C.A.; Jones, C.M.; Martin, M.D.; MacDonald, G.K.; Neenan, P.; Records, A.; Schmale, D.G.; Tateosian, L.; Wei, Q. The persistent threat of emerging plant disease pandemics to global food security. *Proceedings of the National Academy of Sciences* **2021**, *118*, e2022239118, doi:10.1073/pnas.2022239118.
28. Rungjindamai, N.; Jeffries, P.; Xu, X.-M. Epidemiology and management of brown rot on stone fruit caused by *Monilinia laxa*. *European Journal of Plant Pathology* **2014**, *140*, 1-17, doi:10.1007/s10658-014-0452-3.
29. Balsells-Llauradó, M.; Silva, C.J.; Usall, J.; Vall-llaura, N.; Serrano-Prieto, S.; Teixidó, N.; Mesquida-Pesci, S.D.; de Cal, A.; Blanco-Ulate, B.; Torres, R. Depicting the battle between nectarine and *Monilinia laxa*: the fruit developmental stage dictates the effectiveness of the host defenses and the pathogen's infection strategies. *Horticulture Research* **2020**, *7*, 167, doi:10.1038/s41438-020-00387-w.
30. Gramaje, D.; Agustí-Brisach, C.; Pérez-Sierra, A.; Moralejo, E.; Olmo, D.; Mostert, L.; Damm, U.; Armengol, J. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). *Persoonia - Molecular Phylogeny and Evolution of Fungi* **2012**, *28*, 1-13, doi:10.3767/003158512x626155.
31. Browne, G.T. Resistance to Phytophthora Species among Rootstocks for Cultivated Prunus Species. *HortScience* **2017**, *52*, 1471-1476, doi:10.21273/hortsci10391-17.
32. Inderbitzin, P.; Subbarao, K.V. Verticillium Systematics and Evolution: How Confusion Impedes Verticillium Wilt Management and How to Resolve It. *Phytopathology®* **2014**, *104*, 564-574, doi:10.1094/phyto-11-13-0315-ia.
33. Chacón, J.L.; Gramaje, D.; Izquierdo, P.M.; Martínez, J.; Mena, A. Evaluation of six red grapevine cultivars inoculated with *Neofusicoccum parvum*. *European Journal of Plant Pathology* **2020**, *158*, 811-815, doi:10.1007/s10658-020-02111-9.
34. Chacon-Vozmediano, J.L.; Gramaje, D.; Leon, M.; Armengol, J.; Moral, J.; Izquierdo-Canas, P.M.; Martinez-Gascuena, J. Cultivar susceptibility to natural infections caused by fungal

grapevine trunk pathogens in La Mancha Designation of Origin (Spain). *Plants-Basel* **2021**, *10*, 1171, doi:10.3390/plants10061171.
35. Billones-Baaijens, R.; Savocchia, S. A review of *Botryosphaeriaceae* species associated with grapevine trunk diseases in Australia and New Zealand. *Australasian Plant Pathology* **2018**, *48*, 3-18, doi:10.1007/s13313-018-0585-5.
36. Úrbez-Torres, J.; Leavitt, G.; Voegel, T.; Gubler, W. Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. *Plant Disease* **2006**, *90*, 1490-1503.
37. Delgado-Cerrone, L.; Mondino-Hintz, P.; Alaniz-Ferro, S. *Botryospharaceae* species associated with stem canker, die-back and fruit rot on apple in Uruguay. *European Journal of Plant Pathology* **2016**, *146*, 637-655, doi:10.1007/s10658-016-0949-z.
38. Di Francesco, A.; Rusin, C.; Di Foggia, M.; Marceddu, S.; Rombolà, A.; Botelho, R.V.; Baraldi, E. Characterization of apple cultivar susceptibility to *Neofusicoccum parvum* Brazilian strains. *European Journal of Plant Pathology* **2020**, *156*, 939-951, doi:10.1007/s10658-020-01945-7.
39. Mondello, V.; Songy, A.; Battiston, E.; Pinto, C.; Coppin, C.; Trotel-Aziz, P.; Clement, C.; Mugnai, L.; Fontaine, F. Grapevine trunk diseases: A review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Disease* **2018**, *102*, 1189-1217, doi:10.1094/PDIS-08-17-1181-FE.
40. Brown-Rytlewski, D.E.; McManus, P.S. Virulence of *Botryosphaeria dothidea* and *Botryosphaeria obtusa* on apple and management of stem cankers with fungicides. *Plant Disease* **2000**, *84*, 1031-1037, doi:10.1094/pdis.2000.84.9.1031.
41. Zhao, Y.-Q.; Tian, Y.-L.; Wang, L.-M.; Geng, G.-M.; Zhao, W.-J.; Hu, B.-S.; Zhao, Y.-F. Fire blight disease, a fast-approaching threat to apple and pear production in China. *Journal of Integrative Agriculture* **2019**, *18*, 815-820, doi:10.1016/s2095-3119(18)62033-7.
42. Szegedi, E.; Civerolo, E.L. Bacterial diseases of grapevine. *International Journal of Horticultural Science* **2011**, *17*, doi:10.31421/ijhs/17/3/956.
43. Peng, L.; Yang, S.; Zhang, Y.; Haseeb, H.; Song, S.; Xu, X.; Yang, M.; Zhang, J. Characterization and genetic diversity of *Pseudomonas syringae* pv. *syringae* isolates associated with rice bacterial leaf spot in heilongjiang, china. *Biology* **2022**, *11*, 720, doi:10.3390/biology11050720.
44. Galet, P. *Précis de Pathologie Viticole*, 3 ed.; Imprimerie JF Impression: Montpellier, France, 1999; pp. 296.
45. Bois, B.; Zito, S.; Calonnec, A. Climate vs grapevine pests and diseases worldwide: the first results of a global survey. *OENO One* **2017**, *51*, 133-139, doi:10.20870/oeno-one.2017.51.2.1780.
46. Caffarra, A.; Rinaldi, M.; Eccel, E.; Rossi, V.; Pertot, I. Modelling the impact of climate change on the interaction between grapevine and its pests and pathogens: European grapevine moth and powdery mildew. *Agriculture, Ecosystems & Environment* **2012**, *148*, 89-101, doi:10.1016/j.agee.2011.11.017.
47. Kunova, A.; Pizzatti, C.; Saracchi, M.; Pasquali, M.; Cortesi, P. Grapevine powdery mildew: Fungicides for its management and advances in molecular detection of markers associated with resistance. *Microorganisms* **2021**, *9*, 1541, doi:10.3390/microorganisms9071541.
48. Eurostat. *The use of plant protection products in the European Union*; Office for Official Publications of the European Communities: Luxembourg, 2007; pp. 215.
49. Rousseau, J.; Chanfreau, S.; Bontemps, É. *Les cépages résistants and maladies cryptogamiques*; Groupe ICV: Bordeaux, France, 2013; pp. 228.
50. Sambucci, O.; Alston, J.M.; Fuller, K.B.; Lusk, J. The Pecuniary and Nonpecuniary Costs of Powdery Mildew and the Potential Value of Resistant Grape Varieties in California. *American Journal of Enology and Viticulture* **2019**, *70*, 177-187, doi:10.5344/ajev.2018.18032.

51. Vettraino, A.; Potting, R.; Raposo, R. EU legislation on forest plant health: An overview with a focus on *Fusarium circinatum*. *Forests* **2018**, *9*, 568, doi:10.3390/f9090568.
52. Drenkhan, R.; Ganley, B.; Martín-García, J.; Vahalík, P.; Adamson, K.; Adamčíková, K.; Ahumada, R.; Blank, L.; Bragança, H.; Capretti, P., et al. Global geographic distribution and host range of *Fusarium circinatum*, the causal agent of pine pitch canker. *Forests* **2020**, *11*, 724, doi:10.3390/f11070724.
53. Lovat, C.A.; Donnelly, D.J.; Doğmuş-Lehtijärvi, H.T. Mechanisms and metabolomics of the host–pathogen interactions between chestnut (*Castanea* species) and chestnut blight (*Cryphonectria parasitica*). *Forest Pathology* **2019**, *49*, e12562, doi:10.1111/efp.12562.
54. Burgess, T.I.; López-Villamor, A.; Paap, T.; Williams, B.; Belhaj, R.; Crone, M.; Dunstan, W.; Howard, K.; Hardy, G.E.S.J. Towards a best practice methodology for the detection of *Phytophthora* species in soils. *Plant Pathology* **2020**, *70*, 604-614, doi:10.1111/ppa.13312.
55. Benito Garzón, M.; Sánchez de Dios, R.; Sainz Ollero, H. Effects of climate change on the distribution of Iberian tree species. *Applied Vegetation Science* **2008**, *11*, 169-178, doi:10.3170/2008-7-18348.
56. Vivas, M.; Hernández, J.; Corcobado, T.; Cubera, E.; Solla, A. Transgenerational induction of resistance to *Phytophthora cinnamomi* in holm oak. *Forests* **2021**, *12*, 100, doi:10.3390/f12010100.
57. Burgess, T.I.; Scott, J.K.; McDougall, K.L.; Stukely, M.J.C.; Crane, C.; Dunstan, W.A.; Brigg, F.; Andjic, V.; White, D.; Rudman, T.; Arentz, F.; Ota, N.; Hardy, G.E.S.J. Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. *Global Change Biology* **2016**, *23*, 1661-1674, doi:10.1111/gcb.13492.
58. Sánchez, M.E.; Venegas, J.; Romero, M.A.; Phillips, A.J.L.; Trapero, A. *Botryosphaeria* and related taxa causing oak canker in Southwestern Spain. *Plant Disease* **2003**, *87*, 1515-1521, doi:10.1094/pdis.2003.87.12.1515.
59. Grote, U.; Fasse, A.; Nguyen, T.T.; Erenstein, O. Food security and the dynamics of wheat and maize value chains in Africa and Asia. *Frontiers in Sustainable Food Systems* **2021**, *4*, 617009, doi:10.3389/fsufs.2020.617009.
60. Gurung, S.; Hansen, J.M.; Bonman, J.M.; Gironella, A.I.N.; Adhikari, T.B. Multiple disease resistance to four leaf spot diseases in winter wheat accessions from the USDA National Small Grains Collection. *Crop Science* **2012**, *52*, 1640-1650, doi:10.2135/cropsci2011.08.0408.
61. Dweba, C.C.; Figlan, S.; Shimelis, H.A.; Motaung, T.E.; Sydenham, S.; Mwadzingeni, L.; Tsilo, T.J. Fusarium head blight of wheat: Pathogenesis and control strategies. *Crop Protection* **2017**, *91*, 114-122, doi:10.1016/j.cropro.2016.10.002.
62. Pitt, J.I.; Taniwaki, M.H.; Cole, M.B. Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of Food Safety Objectives. *Food Control* **2013**, *32*, 205-215, doi:10.1016/j.foodcont.2012.11.023.
63. Martins, P.M.M.; Merfa, M.V.; Takita, M.A.; De Souza, A.A. Persistence in phytopathogenic bacteria: Do we know enough? *Front Microbiol* **2018**, *9*, 1099, doi:10.3389/fmicb.2018.01099.
64. Xin, X.-F.; Kvitko, B.; He, S.Y. *Pseudomonas syringae*: What it takes to be a pathogen. *Nature Reviews Microbiology* **2018**, *16*, 316-328, doi:10.1038/nrmicro.2018.17.
65. Hikichi, Y.; Wali, U.M.; Ohnishi, K.; Kiba, A. Mechanism of disease development caused by a multihost plant bacterium, *Pseudomonas cichorii*, and its virulence diversity. *Journal of General Plant Pathology* **2013**, *79*, 379-389, doi:10.1007/s10327-013-0461-7.
66. Nakayinga, R.; Makumi, A.; Tumuhaise, V.; Tinzaara, W. *Xanthomonas* bacteriophages: a review of their biology and biocontrol applications in agriculture. *BMC Microbiology* **2021**, *21*, 291, doi:10.1186/s12866-021-02351-7.
67. Peritore-Galve, F.C.; Tancos, M.A.; Smart, C.D. Bacterial canker of tomato: Revisiting a global and economically damaging seedborne pathogen. *Plant Disease* **2021**, *105*, 1581-1595, doi:10.1094/pdis-08-20-1732-fe.

68. Mansfield, J.; Genin, S.; Magori, S.; Citovsky, V.; Sriariyanum, M.; Ronald, P.; Dow, M.A.X.; Verdier, V.; Beer, S.V.; Machado, M.A.; Toth, I.A.N.; Salmond, G.; Foster, G.D. Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 614-629, doi:10.1111/j.1364-3703.2012.00804.x.
69. Dupuis, B.; Nkuriyingoma, P.; Van Gijsegem, F. Economic impact of *Pectobacterium* and *Dickeya* species on potato crops: A Review and case study. In *Plant diseases caused by Dickeya and Pectobacterium species*, Van Gijsegem, F., van der Wolf, J.M., Toth, I.K., Eds. Springer International Publishing: Cham, 2021; 10.1007/978-3-030-61459-1_8pp. 263-282.
70. Chehri, K.; Salleh, B.; Yli-Mattila, T.; Reddy, K.; Abbasi, S. Molecular characterization of pathogenic *Fusarium* species in cucurbit plants from Kermanshah province, Iran. *Saudi J. Biol. Sci.* **2011**, *18*, 341-351, doi:10.1016/j.sjbs.2011.01.007.
71. González, V.; Aguado, A.; Garcés-Claver, A. First report of *Fusarium oxysporum* causing wilt and root rot in common borage (*Borago officinalis*) in Spain. *Plant Disease* **2019**, *103*, 1774-1774, doi:10.1094/pdis-02-19-0259-pdn.
72. Keinath, A.P.; Wechter, W.P.; Rutter, W.B.; Agudelo, P.A. Cucurbit rootstocks resistant to *Fusarium oxysporum* f. sp. *niveum* remain resistant when coinfected by *Meloidogyne incognita* in the field. *Plant Disease* **2019**, *103*, 1383-1390, doi:10.1094/PDIS-10-18-1869-RE.
73. Srinivas, C.; Nirmala Devi, D.; Narasimha Murthy, K.; Mohan, C.D.; Lakshmeesha, T.R.; Singh, B.; Kalagatur, N.K.; Niranjana, S.R.; Hashem, A.; Alqarawi, A.A.; Tabassum, B.; Abd_Allah, E.F.; Chandra Nayaka, S.; Srivastava, R.K. *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity–A review. *Saudi J. Biol. Sci.* **2019**, *26*, 1315-1324, doi:10.1016/j.sjbs.2019.06.002.
74. Shukla, A.; Sharma, D.; Sharma, M.; Tarafdar, A.; Gupta, M. First report of *Fusarium equiseti* causing crown and root rot of cucumber in India. *Journal of Plant Pathology* **2022**, *104*, 875-875, doi:10.1007/s42161-022-01075-5.
75. Sandoval-Denis, M.; Lombard, L.; Crous, P. Back to the roots: a reappraisal of *Neocosmospora*. *Persoonia-Molecular Phylogeny and Evolution of Fungi* **2019**, *43*, 90-185, doi:10.3767/persoonia.2019.43.04.
76. González, V.; Ruiz, J.; Picó, B.; García-Martínez, S.; Garcés-Claver, A.; Flores-León, A. First report of *Neocosmospora falciformis* causing wilt and root rot of muskmelon in Spain. *Plant Disease* **2020**, *104*, 4, doi:10.1094/PDIS-09-19-2013-PDN.
77. González, V.; García-Martínez, S.; Flores-León, A.; Ruiz, J.; Picó, B.; Garcés-Claver, A. *Neocosmospora keratoplastica*, a relevant human fusarial pathogen is found to be associated with wilt and root rot of muskmelon and watermelon crops in Spain: epidemiological and molecular evidences. *European journal of plant pathology* **2020**, *156*, 1189-1196, doi:10.1007/s10658-020-01931-z.
78. de Sousa Linhares, C.M.; Ambrósio, M.M.Q.; Castro, G.; Torres, S.B.; Esteras, C.; de Sousa Nunes, G.H.; Picó, B. Effect of temperature on disease severity of charcoal rot of melons caused by *Macrophomina phaseolina*: implications for selection of resistance sources. *European Journal of Plant Pathology* **2020**, *158*, 431-441, doi:10.1007/s10658-020-02083-w.
79. Cohen, R.; Elkabetz, M.; Paris, H.S.; Freeman, S.; Gur, A. Charcoal rot (*Macrophomina phaseolina*) across melon diversity: evaluating the interaction between the pathogen, plant age and environmental conditions as a step towards breeding for resistance. *European Journal of Plant Pathology* **2022**, 10.1007/s10658-022-02500-2, 1-13, doi:10.1007/s10658-022-02500-2.
80. Gondal, A.S.; Rauf, A.; Naz, F. Anastomosis groups of *Rhizoctonia solani* associated with tomato foot rot in Pothohar Region of Pakistan. *Scientific Reports* **2019**, *9*, 3910, doi:10.1038/s41598-019-40043-5.
81. Troncoso-Rojas, R.; Tiznado-Hernández, M.E. *Alternaria alternata* (black rot, black spot). In *Postharvest decay: Control strategies*, Bautista-Baños, S., Ed. Academic Press: London, 2014; 10.1016/b978-0-12-411552-1.00005-3pp. 147-187.

82. Rahman, M.; Suzuki, K.; Islam, M.; Dey, T.; Harada, N.; Hossain, D. Molecular characterization, mycelial compatibility grouping, and aggressiveness of a newly emerging phytopathogen, *Sclerotinia sclerotiorum*, causing white mold disease in new host crops in Bangladesh. *Journal of Plant Pathology* **2020**, *102*, 775-785, doi:10.1007/s42161-020-00503-8.
83. Dean, R.; Van Kan, J.A.L.; Pretorius, Z.A.; Hammond-Kosack, K.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J.; Foster, G.D. The top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 414-430, doi:10.1111/j.1364-3703.2011.00783.x.
84. Petrasch, S.; Knapp, S.J.; van Kan, J.A.L.; Blanco-Ulate, B. Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea*. *Mol. Plant Pathol.* **2019**, *20*, 877-892, doi:10.1111/mpp.12794.
85. Dowling, M.; Peres, N.; Villani, S.; Schnabel, G. Managing *Colletotrichum* on fruit crops: A "complex" challenge. *Plant Disease* **2020**, *104*, 2301-2316, doi:10.1094/pdis-11-19-2378-fe.
86. Sangpueak, R.; Phansak, P.; Buensanteai, N. Morphological and molecular identification of *Colletotrichum* species associated with cassava anthracnose in Thailand. *Journal of Phytopathology* **2017**, *166*, 129-142, doi:10.1111/jph.12669.
87. Salotti, I.; Ji, T.; Rossi, V. Temperature requirements of *Colletotrichum* spp. belonging to different clades. *Frontiers in Plant Science* **2022**, *13*, 953760, doi:10.3389/fpls.2022.953760.
88. Arendrup, M.C.; Cuenca-Estrella, M.; Lass-Flörl, C.; Hope, W. EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). *Clin. Microbiol. Infect.* **2012**, *18*, E246-E247, doi:10.1111/j.1469-0691.2012.03880.x.
89. CLSI. CLSI standard M07 - Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (11th ed). Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.
90. Matheron, M.; Mircetich, S. Seasonal variation in susceptibility of *Juglans hindsii* and paradox rootstocks of English walnut trees to *Phytophthora citricola*. *Phytopathology* **1985**, *75*, 970-972.
91. Álvarez Bernaola, L.A. Estudios de etiología, epidemiología y control de un nuevo síndrome de lesiones en tronco y ramas principales de cítricos asociado a *Phytophthora*. Universitat Politècnica de València, Valencia, Spain, 2008.
92. Perczak, A.; Gwiazdowska, D.; Gwiazdowski, R.; Juś, K.; Marchwińska, K.; Waśkiewicz, A. The inhibitory potential of selected essential oils on *Fusarium* spp. growth and mycotoxins biosynthesis in maize seeds. *Pathogens* **2020**, *9*, 23.
93. Abd-El-Khair, H.; Abdel-Gaied, T.G.; Mikhail, M.S.; Abdel-Alim, A.I.; El-Nasr, H.I.S. Biological control of *Pectobacterium carotovorum* subsp. *carotovorum*, the causal agent of bacterial soft rot in vegetables, in vitro and in vivo tests. *Bulletin of the National Research Centre* **2021**, *45*, 37, doi:10.1186/s42269-021-00491-4.
94. Hernández-Muñoz, P.; Almenar, E.; Valle, V.D.; Velez, D.; Gavara, R. Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria×ananassa*) quality during refrigerated storage. *Food Chem.* **2008**, *110*, 428-435, doi:10.1016/j.foodchem.2008.02.020.
95. Romanazzi, G.; Feliziani, E.; Santini, M.; Landi, L. Effectiveness of postharvest treatment with chitosan and other resistance inducers in the control of storage decay of strawberry. *Postharvest Biol. Technol.* **2013**, *75*, 24-27, doi:10.1016/j.postharvbio.2012.07.007.
96. Riquelme, D.; Aravena, Z.; Valdés-Gómez, H.; Latorre, B.A.; Díaz, G.A.; Zoffoli, J.P. Characterization of *Botrytis cinerea* and *B. prunorum* from healthy floral structures and decayed 'Hayward' kiwifruit during post-harvest storage. *Plant Disease* **2021**, *105*, 2129-2140, doi:10.1094/pdis-04-20-0878-re.

97. Pereira, L.; Cunha, A.; Almeida-Aguiar, C. Portuguese propolis from Caramulo as a biocontrol agent of the apple blue mold. *Food Control* **2022**, *139*, 109071, doi:10.1016/j.foodcont.2022.109071.
98. Loebler, M.; Sánchez, C.; Muchagato Maurício, E.; Diogo, E.; Santos, M.; Vasilenko, P.; Cruz, A.S.; Mendes, B.; Gonçalves, M.; Duarte, M.P. Potential application of propolis extracts to control the growth of *Stemphylium vesicarium* in "Rocha" pear. *Applied Sciences* **2020**, *10*, 1990, doi:10.3390/app10061990.
99. Onaran, A.; Yanar, Y. In vivo and in vitro antifungal activities of five plant extracts against various plant pathogens. *Egyptian Journal of Biological Pest Control* **2016**, *26*, 405-411.
100. Sánchez-Hernández, E.; González-García, V.; Martín-Gil, J.; Lorenzo-Vidal, B.; Palacio-Bielsa, A.; Martín-Ramos, P. Phytochemical screening and antibacterial activity of *Taxus baccata* L. against *Pectobacterium* spp. and *Dickeya chrysanthemi*. *Horticulturae* **2023**, *9*, 201.
101. Wang, C.; Yuan, S.; Zhang, W.; Ng, T.; Ye, X. Buckwheat antifungal protein with biocontrol potential to inhibit fungal (*Botrytis cinerea*) infection of cherry tomato. *J. Agric. Food. Chem.* **2019**, *67*, 6748-6756, doi:10.1021/acs.jafc.9b01144.
102. de León, L.; Siverio, F.; López, M.M.; Rodríguez, A. Comparative efficiency of chemical compounds for *in vitro* and *in vivo* activity against *Clavibacter michiganensis* subsp. *michiganensis*, the causal agent of tomato bacterial canker. *Crop Protection* **2008**, *27*, 1277-1283, doi:10.1016/j.cropro.2008.04.004.
103. Martín Sanz, A.; Caminero Saldaña, C.; Pérez de la Vega, M. Bacteriosis en guisante (*Pisum sativum* L.): situación en Castilla y León, caracterización de los patógenos implicados y búsqueda de fuentes de resistencia. Universidad de León, León, 2008.
104. European Food Safety Authority. Conclusion on the peer review of the pesticide risk assessment of the active substance azoxystrobin. *EFSA Journal* **2010**, *8*, 1542, doi:10.2903/j.efsa.2010.1542.
105. Zubrod, J.P.; Bundschuh, M.; Arts, G.; Brühl, C.A.; Imfeld, G.; Knäbel, A.; Payraudeau, S.; Rasmussen, J.J.; Rohr, J.; Scharmüller, A.; Smalling, K.; Stehle, S.; Schulz, R.; Schäfer, R.B. Fungicides: An overlooked pesticide class? *Environmental Science & Technology* **2019**, *53*, 3347-3365, doi:10.1021/acs.est.8b04392.
106. Piszczek, J.; Pieczul, K.; Kiniec, A. First report of G143A strobilurin resistance in *Cercospora beticola* in sugar beet (*Beta vulgaris*) in Poland. *Journal of Plant Diseases and Protection* **2017**, *125*, 99-101, doi:10.1007/s41348-017-0119-3.
107. Emara, A.R.; Ibrahim, H.M.; Masoud, S.A. The role of storage on Mancozeb fungicide formulations and their antifungal activity against *Fusarium oxysporium* and *Rhizoctonia solani*. *Arabian Journal of Chemistry* **2021**, *14*, 103322, doi:10.1016/j.arabjc.2021.103322.
108. Cocco, P. Time for re-evaluating the human carcinogenicity of ethylenedithiocarbamate fungicides? A systematic review. *International Journal of Environmental Research and Public Health* **2022**, *19*, 2632, doi:10.3390/ijerph19052632.
109. Dann, E.; McLeod, A. Phosphonic acid: a long-standing and versatile crop protectant. *Pest Management Science* **2020**, *77*, 2197-2208, doi:10.1002/ps.6156.
110. Gómez-Merino, F.C.; Trejo-Téllez, L.I. Biostimulant activity of phosphite in horticulture. *Scientia Horticulturae* **2015**, *196*, 82-90, doi:10.1016/j.scienta.2015.09.035.
111. Fournier, B.; Pereira Dos Santos, S.; Gustavsen, J.A.; Imfeld, G.; Lamy, F.; Mitchell, E.A.D.; Mota, M.; Noll, D.; Planchamp, C.; Heger, T.J. Impact of a synthetic fungicide (fosetyl-Al and propamocarb-hydrochloride) and a biopesticide (*Clonostachys rosea*) on soil bacterial, fungal, and protist communities. *Sci. Total Environ.* **2020**, *738*, 139635, doi:10.1016/j.scitotenv.2020.139635.
112. Walton, V.M.; Pringle, K.L. Effects of pesticides and fungicides used on grapevines on the mealybug predatory beetle *Nephus 'boschianus'* (Coccinellidae, Scymnini). *South African Journal of Enology & Viticulture* **2017**, *22*, 107-110, doi:10.21548/22-2-2204.

ANNEX: PATENTS

PATENT 1: "Compuesto reticulado de lignina metacrilada y oligómeros de quitosano capaz de actuar como nanotransportador de compuestos bioactivos, método de obtención y usos". Spanish patent with application number P202131019, filed on 29th October 2021. Granted on 3rd May 2023, with patent number ES2940132.

MINISTERIO
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Justificante de presentación electrónica de solicitud de patente

Este documento es un justificante de que se ha recibido una solicitud española de patente por vía electrónica utilizando la conexión segura de la O.E.P.M. De acuerdo con lo dispuesto en el art.14.3 del Reglamento para la ejecución de la Ley 11/1986, de 20 de marzo, de Patentes, se han asignado a su solicitud un número de expediente y una fecha de recepción de forma automática. La fecha de presentación de la solicitud a la que se refiere el art. 22 de la Ley le será comunicada posteriormente.

Número de solicitud:	P202131019
Fecha de recepción:	29 octubre 2021, 10:42 (CEST)
Oficina receptora:	OEPM Madrid

PATENT 2: "Nanomaterial basado en el autoensamblaje de g-C_3N_4 y oligómeros de quitosano, proceso de obtención y usos". Spanish patent with application number P202230668, filed on 20th July 2022. Extended with international application number PCT/ES2023/070409.

GOBIERNO DE ESPAÑA
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Justificante de presentación electrónica de solicitud de patente

Este documento es un justificante de que se ha recibido una solicitud española de patente por vía electrónica utilizando la conexión segura de la O.E.P.M. De acuerdo con lo dispuesto en el art. 16.1 del Reglamento de ejecución de la Ley 24/2015 de Patentes, se han asignado a su solicitud un número de expediente y una fecha de recepción de forma automática. La fecha de presentación de la solicitud a la que se refiere el art. 24 de la Ley le será comunicada posteriormente.

Número de solicitud:	P202230668
Fecha de recepción:	20 julio 2022, 12:33 (CEST)
Oficina receptora:	OEPM Madrid

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TRATADO DE COOPERACIÓN EN MATERIA DE PATENTES NOTIFICACIÓN DE LA RECEPCIÓN DE LOS DOCUMENTOS QUE CONSTITUYEN SUPUESTAMENTE UNA SOLICITUD INTERNACIONAL PRESENTADA DE FORMA ELECTRÓNICA.

(Instrucciones Administrativas del PCT, Parte Séptima)

1.-Se notifica al solicitante que la Oficina Receptora ha recibido en la fecha de recepción indicada más abajo, los documentos que supuestamente constituyen una solicitud internacional.

2.-Se llama la atención del solicitante sobre el hecho de que la Oficina Receptora no ha comprobado aún si estos documentos satisfacen las condiciones del art. 11.1, es decir, si cumple los requisitos para que le sea atribuida una fecha de presentación internacional. En cuanto la Oficina Receptora haya comprobado los documentos, avisará al solicitante.

3.-El número de la supuesta solicitud internacional indicado más abajo ha sido otorgado automáticamente a estos documentos. Se invita al solicitante a mencionar este número en toda la correspondencia con la Oficina Receptora.

Número de presentación	300486618
Solicitud Número PCT	PCT/ES2023/070409
Fecha de recepción	23 junio 2023
Oficina Receptora	Oficina Española de Patentes y Marcas, Madrid

PATENT 3: "Nanomaterial encapsulante formado por g-C_3N_4 y oligómeros de quitosano enlazados a hidroxiapatito, proceso de obtención y usos". Spanish patent with application number P202330435, filed on 31st May 2023.

GOBIERNO DE ESPAÑA
MINISTERIO DE INDUSTRIA, COMERCIO Y TURISMO
Oficina Española de Patentes y Marcas

Justificante de presentación electrónica de solicitud de patente

Este documento es un justificante de que se ha recibido una solicitud española de patente por vía electrónica utilizando la conexión segura de la O.E.P.M. De acuerdo con lo dispuesto en el art. 16.1 del Reglamento de ejecución de la Ley 24/2015 de Patentes, se han asignado a su solicitud un número de expediente y una fecha de recepción de forma automática. La fecha de presentación de la solicitud a la que se refiere el art. 24 de la Ley le será comunicada posteriormente.

Número de solicitud:	P202330435
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Oficina receptora:	OEPM Madrid