SCHOOL OF ENGINEERING, ILHA SOLTEIRA
DEPARTMENT OF PHYSICS AND CHEMISTRY
POSTGRADUATION PROGRAM IN MATERIALS SCIENCE

PEDRO HENRIQUE CORREIA DE LIMA

Lignin nanoparticles as sustainable carriers of herbicide: from development to effects in plants

Ilha Solteira – SP

2025

POSTGRADUATION PROGRAMM OF MATERIALS SCIENCE

PEDRO HENRIQUE CORREIA DE LIMA

Lignin nanoparticles as sustainable carriers of herbicide: from development to effects in plants

Thesis submitted to the Postgraduation Program in Materials Science of the Department of Physics and Chemistry of the São Paulo State University "Júlio de Mesquita Filho", School of Engineering, Ilha Solteira, as a requirement to obtain the title of Doctor in Materials Science.

Concentration area: Materials chemistry
Advisor: Prof. Dr. Renato Grillo

FICHA CATALOGRÁFICA
Desenvolvido pelo Serviço Técnico de Biblioteca e Documentação

L732l   Lima, Pedro Henrique Correia de.
Lignin nanoparticles as sustainable carriers of herbicide: from development to effects in plants / Pedro Henrique Correia de Lima. -- Ilha Solteira: [s.n.], 2025
100 f. : il.

Tese (doutorado) - Universidade Estadual Paulista. Faculdade de Engenharia de Ilha Solteira. Área de conhecimento: Química dos Materiais , 2025

Orientador: Renato Grillo

Inclui bibliografia

1. Lignin. 2. Nanoherbicides. 3. Atrazine. 4. Plants. 5. Sustainable.

Amanda Sertori dos Santos
Bibliotecária - CRB/8-9061
Seção Técnica de Referência, Atendimento ao
Usuário e Documentação
Diretoria Técnica de Biblioteca e Documentação

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Ilha Solteira

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: NANOPARTÍCULAS DE LIGNINA COMO CARREADORES SUSTENTÁVEIS DE HERBICIDA: DO DESENVOLVIMENTO AOS EFEITOS EM PLANTAS

AUTOR: PEDRO HENRIQUE CORREIA DE LIMA
ORIENTADOR: RENATO GRILLO

Aprovado como parte das exigências para obtenção do Título de Doutor em Ciência dos Materiais, área: Química dos Materiais pela Comissão Examinadora:

gov.br Documento assinado digitalmente
RENATO GRILLO
Data: 30/04/2025 18:07:18-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. RENATO GRILLO (Participaçao Presencial)
Departamento de Fisica e Quimica / Faculdade de Engenharia de Ilha Solteira - UNESP

gov.br Documento assinado digitalmente
MARCELO CARVALHO MINHOTO TEIXEIRA FILHO
Data: 30/04/2025 17:59:39-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. MARCELO CARVALHO MINHOTO TEIXEIRA FILHO (Participaçao Presencial)
Departamento de Fitossanidade, Engenharia Rural e Solos / Faculdade de Engenharia de Ilha Solteira - UNESP

gov.br Documento assinado digitalmente
ALEX OTAVIO SANCHES
Data: 29/04/2025 17:24:41-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. ALEX OTÁVIO SANCHES (Participaçao Presencial)
Departamento de Física e Química / Faculdade de Engenharia de Ilha Solteira - UNESP

gov.br Documento assinado digitalmente
EMILIO DE CASTRO MIGUEL
Data: 30/04/2025 12:02:24-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. EMÍLIO DE CASTRO MIGUEL (Participaçao Virtual)
Departamento de Engenharia Metalúrgica e de Materias / Universidade Federal do Ceará - UFC

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Ilha Solteira

Profa. Dra. MARIA EUGENIA TAVERNA (Participaçao Virtual)
Departamento de Ingeniería Química / Universidad Tecnológica Nacional - Facultad Regional San Francisco

Ilha Solteira, 28 de abril de 2025

LIST OF ABREVIATIONS AND ACRONYMS

μ	Chemical potential
AL	Alkali Lignin
ATZ	Atrazine
BB	Bibenzyl
C	Concentration
CA	Citric acid
CAT	Catalase
CMC	Carboxymethyl cellulose
CNC	Cellulose nanocrystals
CNFs	Cellulose nanofibers
DCMC	Dialdehyde carboxymethyl cellulose
DFT	Density functional theory
DLS	Dynamic Light Scattering
DTT	Dithiothreitol
EDS	Energy dispersive spectroscopy
EE	Encapsulation efficiency
ENPs	Engineered nanoparticles
FCI	Falker chlorophyll index
FTIR	Fourier transform infrared spectroscopy
GADPH	Glyceraldehyde 3-phosphate dehydrogenase
GLY	Glycine methyl ester
GMA	Glycidyl methacrylate
GST	Glutathione S-transferases
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HOMO	Highest occupied molecular orbital
I_2	Iodine

K Release rate Constant

KBr Potassium Bromide

KI Iodine Potassium

LDH Layered double hydroxides

LUMO Lowest unoccupied molecular orbital

MDA Malondialdehyde

Mo Mass of atrazine in the interval t

MTZ Metribuzin

η Chemical hardness

NBT Nitro Blue Tetrazolium

NTA Nanoparticles tracking analysis

PCL Polycaprolactone

PdI Polydispersity Index

PEG Polyethyleneglycol

PER Peroxidase

pH Hydrogen potential

PHFBA Poly 2,2,3,4,4,4-hexafluorobutyl methacrylate

PHR Phosphate starvation response

POD Guaiacol peroxidase

PPE 2- phenethyl phenyl ether

PVP Polyvinylpyrrolidone

ROS Reactive oxygen species

RT-qPCR Reverse transcription - quantitative polymerase chain reaction

S Chemical softness

SDGs Sustainable Development Goals

SEM Scanning electron microscopy

SEM/EDS Scanning Electron Microscopy with coupled energy dispersive spectroscopy

SLNPs Spherical lignin nanoparticles

SLNPs_ATZ Spherical lignin nanoparticles with atrazine

SOD	Superoxide dismutase
t	Time
TCA	Thiobarbituric acid
Td	Degradation temperature
TGA	Thermogravimetric analysis
UN	United Nations
UV-Vis	Ultraviolet-visible
Wf	Total weight
Wi	Initial weight
Ω	Electrophilicity index

ACKNOWLEGDMENTS

Gratefulness is a social responsibility, to make people happy and ignite the lightness in an exchange of gestures that touch magic. Being grateful for my mother is to be close to God. For my father, gratefulness comes with his affectionate gestures. Having them both is enough for me to be grateful.

To my dear grandmother Luiza, I will always remember you wherever I go.

I imagine that my brothers (Ana Rúbia, Natália and João) would be upset if I were not grateful for their existence and all their wisdom. I also imagine that my nephews: Osmar, Luiz and Maria Júlia will not even know that they were written here, but they are dearest in my eternity.

Throughout my academic journey, there were people who shaped me into who I am today. Academically: Suédina, Albaniza, Cristiano, Renato Ribeiro-Viana. Personally: Maria Luanna, Maria Vitorino, Giovanni, Isaul, Paula Roberta, Rogério, Tatiane, Maria Clara and Flávio.

I want to express gratitude to my advisor, Prof. Dr. Renato Grillo for his guidance and knowledge.

And extend to the Laboratory of Environmental Nanochesmitry (LNA) for helping me with their knowledge and collaboration.

Also, I would like to thank Loane, Patrícia and Fábio for all their technical support.

To the São Paulo State University Professors and staff from Physics and Chemistry Department of Ilha Solteira.

To the Molecular Phytopathology Laboratory, especially Prof. Dr. Paulo Cezar Ceresini and Prof. Dr. Gabriel Madoglio Favara for their assistance with analyses.

To CAPES for the scholarship granting. Additionally, this work received support from FAPESP (#2022/03219-2, #2023/00541-3, #2023/17369-9, #2021/03402-9) and CNPq (#310846/2022-6). The present work was carried out with the support from the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financial Code 001.

RESUMO

Por décadas, os agricultores têm tentado controlar as plantas daninhas e aumentar a produtividade das colheitas para fornecer alimentos para uma crescente população através do uso de herbicidas. A modernidade através da nanociência auxilia a diminuição da quantidade dos herbicidas através de nanocarreadores inorgânicos, híbridos e orgânicos (ou nanoherbicidas) no campo experimental. Nesse contexto, nanocarreadores orgânicos provindos de material lignocelulósico surgem como candidatos promissores, destacando-se a lignina: encontrada em plantas, é um resíduo agrícola cuja estrutura polifenólica pode formar (núcleos hidrofóbicos/cascas hidrofílicas) nanoestruturas que podem interagir com herbicidas como a atrazina e liberar lentamente esse bioativo. Assim, a presente Tese teve como objetivo principal discutir sobre nanocarreadores constituídos de materiais lignocelulósicos para liberação de agroquímicos, bem como desenvolver nanopartículas esféricas de lignina (SLNPs) com o herbicida atrazina (SLNPs_ATZ) a fim de se compreender os seus efeitos em organismos alvo e não alvo. As nanopartículas foram fabricadas pelo método de precipitação antissolvente e caracterizadas por TGA, FTIR, MEV/EDS, DLS, NTA, espectroscopia UV-VIS. SLNPs apresentaram morfologia esférica e distribuição de tamanho de 145 ± 45 ~ 178.9 ± 60.88 nm sem ATZ e 136 ± 43 ~ 268.2 ± 69.58 nm com ATZ e eficiência de encapsulação de 74,2 %. Testes de liberação foram feitos por espectroscopia UV-VIS e observou-se uma liberação de 43,26% de ATZ após 168 horas para SLNPs_ATZ, sendo Korsmeyes-peppas o modelo de liberação encontrado. Em paralelo, a Teoria do Funcional da Densidade (DFT) foi aplicada entre a atrazina e duas moléculas modelo de lignina apresentando energias de gap mais baixas quando as moléculas foram colocadas juntas, indicando uma forte interação entre lignina e atrazina. Além disso, o efeito das SLNPs com e sem ATZ foi avaliado em organismos não alvo. Assim, experimentos em hidroponia (escolhidos como meio inerte) foram realizados em alfaces lisas (*Lactuca sativa var. capitata*). Uma dose subletal de atrazina (30 µg/L) foi usada para manter as alfaces vivas por 28 dias a fim de observar potenciais alterações fisiológicos e bioquímicos nas plantas. Alfaces tratadas com SLNPs e SLNPs_ATZ não apresentaram alterações significativas no comprimento da raiz e na parte aérea quando comparadas ao controle, enquanto alfaces tratadas com ATZ apresentaram. Além disso, testes bioquímicos como enzimáticos (catalase-CAT, peroxidase de guaiacol - POD, superóxido dismutase - SOD e peroxidação lipídica - MDA) e expressão relativa de genes foram avaliados. MDA e CAT mostraram diferenças significativas comparando ATZ com todos os outros tratamentos. Pela expressão gênica, os genes CAT1 expressaram mais alto nas alfaces tratadas só SLNPs e ATZ pura, e os genes GST6 tiveram uma expressão mais alta com SLNPs. No entanto, alfaces tratadas com SLNPs apresentaram mais similaridades fisiológicas ao controle, indicando uma possível tolerância de alfaces quando interagem com SLNPs. Além disso, os genes PER51 apresentaram uma repressão em plantas tratadas com SLNPs_ATZ e ATZ (dano), e nenhuma alteração significativa com SLNPs quando comparado ao controle. Finalmente, sementes/mudas de plantas daninhas tiveram possíveis interferências na germinação/desenvolvimento quando tratadas com o nanoherbicida. Indicando a lignina como candidata sustentável para o preparo de nanocarreadores de herbicida, contribuindo para a. melhora da atividade herbicida em plantas, uma vez que mostrou possíveis alterações na expressão gênica e controle de plantas daninhas.

Palavras-chave: lignina, nanoherbicidas, atrazina, sustentável, plantas não-alvo e alvo.

ABSTRACT

For decades, farmers have been trying to control weeds and increase crop productivity to provide food for a growing population through the usage of herbicides, and nanoscience has been helping to reduce the quantity of herbicides through inorganic, hybrid, and organic nanocarriers (or nanoherbicides) in the experimental field. In this context, lignocellulosic organic nanocarriers emerge as promising candidates, and lignin standing out: found in plants, it is an agricultural residue whose polyphenolic structure can form (hydrophobic cores/hydrophilic shells) nanostructures that may interact with herbicides such as atrazine and slowly release this bioactive. Thus, the main objective of this thesis was to discuss nanocarriers made of lignocellulosic materials for agrochemicals release as well as to develop spherical lignin nanoparticles (SLNPs) with the herbicide atrazine (SLNPs_ATZ) to understand their effects on target and non-target organisms. The nanoparticles were manufactured by an antisolvent precipitation method and characterized by TG, FTIR, SEM/EDS, DLS, NTA, and UV-VIS spectroscopy. SLNPs presented spherical morphology and size distribution of 145 ± 45 ~ 178.9 ± 60.88 nm without ATZ, 136 ± 43 ~ 268.2 ± 69.58 nm with ATZ and loading efficiency of 74.2%. Release tests were performed by UV-VIS spectroscopy and a release of 43.26% of ATZ was found after 168 hours, with Korsmeyes-peppas as the release model. In parallel, Density Functional Theory (DFT) was applied among atrazine and two model lignin molecules and showed lower band gap energies when atrazine and lignin were placed together, indicating a strong interaction between molecules. Furthermore, the effect of SLNPs was evaluated in non-target organisms at hydroponic environment (chosen as inert medium) and lettuces (Lactuca sativa var. capitata) were selected to be the plant model. Sublethal doses of atrazine (30 µg/L) were managed to keep lettuces alive for 28 days and understand physiological and biochemical parameters. Lettuces treated with SLNPs and SLNPs_ATZ did not show significant changes in root and shoot length when compared to the control while lettuces treated with ATZ did. Then, biochemical tests such as enzymatic (catalase-CAT, guaiacol peroxidase - POD, superoxide dismutase - SOD, and lipid peroxidation - MDA) and relative gene expression were evaluated where MDA and CAT showed significant differences comparing ATZ with all other treatments. By gene expression, the CAT1 genes expressed higher in lettuces treated with SLNPs and ATZ, and the GST6 genes had a higher expression in those treated with SLNPs. However, lettuces treated with SLNPs showed less physiological changes compared to the control, indicating a possible tolerance of lettuce when interacting with SLNPs. In addition, the PER51 genes showed repression in plants treated with SLNPs_ATZ and ATZ (damage), and no significant change with SLNPs when compared to the control. Finally, weed seeds/seedlings had possible interferences in germination/development by nanoherbicide, indicating lignin as a sustainable molecule that in nanosphere forms can load atrazine and be effectively used in future experiments with non-target/target plants, showing possible results in gene expression and weed control.

Keywords: lignin, nanoherbicides, atrazine, sustainable, non-target and target plants.

SUMARY

GENERAL INTRODUCTION

The demand to increase the amount of food with sustainable processes while facing rapid population growth leads farmers and industries to search for new eco-friendly tools able to enhance harvest quality and reach the Sustainable Development Goals (SDG). Mainly, in the combat against weeds, plants unwilled in large crops (Darbyshire et al., 2024; Aijaz et al., 2025). Weeds disturb other plants by space, nutrients, water and light competition, reducing up to 50% yield production (Parven et al., 2024). In this scenario, herbicides are used to modify physiological processes in plants, acting in their metabolic pathways, and causing their death or interrupting their growth (Ghazi et al., 2023). Although, herbicides are small molecules that may not fulfill their goals and be leached, driven away, or drained, harming non-target species and humans. Besides, weeds are resisting to this agrochemical, urging new agricultural implements (Perotti et al., 2020; Yu et al., 2021). A great biotechnology potential comes from nanotechnology, bringing to materials new physical, chemical and biological characteristics (Thakur and Yadav, 2025).

Nanoparticles (structures with a nanometric size or a property) offer a higher superficial area and allow nanoherbicides to have a better interaction with plants alongside superficial charge, size, composition, solubility, and fabrication method (Jogaiah et al., 2021, Grillo et al., 2021a; Lima et al., 2022), developing focused and intelligent systems. Nanoherbicides can present vantages: delay on active degradation (Cheng et al., 2024), reduced toxicity on human cells (Dong et al., 2021a), alteration of bioactive in soil lixiviation (Kah et al., 2014) and increase herbicide activity (Oliveira et al., 2015; Pontes et al., 2021). Moreover, they have organic, inorganic and hybrid origin, highlighting the organic (or soft) nanomaterials (Forini et al. 2022) due to their biodegradability that can minimize harmful risks to non-target organisms and the ecosystem (Kusumavathi, et al., 2024).

Although some organic nanoherbicides can be fabricated through synthetic materials (Mattos et al., 2017). The natural organic nanoherbicides with lignocellulosic background have been rising in research due to their biodegradability and safe production (Chamundeeswari et al., 2019; Shrestha et al., 2021; Zhang et al., 2019; Bhattacharyya., 2020; Ur Rahim et al., 2021). Furthermore, lignocellulosic materials are obtained by agricultural waste, reinforcing the use from plant to plant, ideal scenario for a circular bioeconomy (Lima et al., 2021; Vinzant et al., 2025).

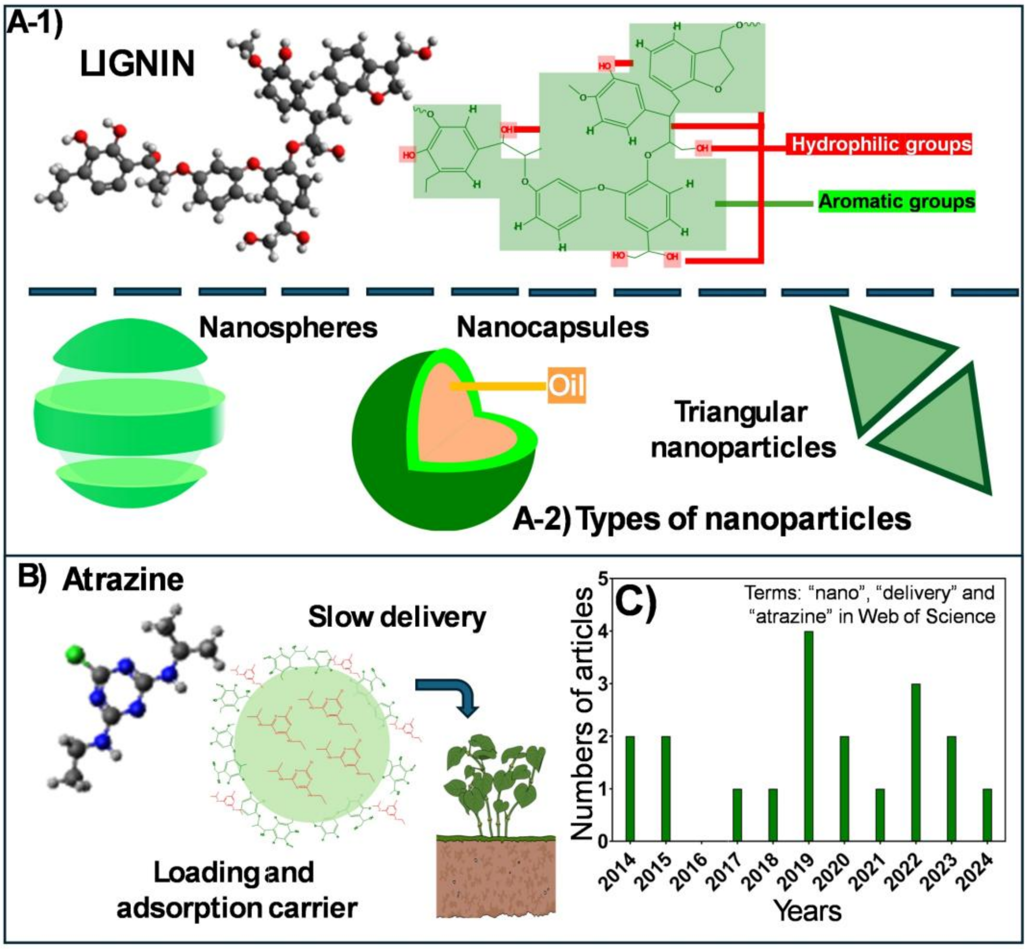


Figure 1 – (a-1) Lignin structure; (a-2) Types of lignin nanoparticles; (b) atrazine and nanospheres with a slow-release representation in plants, and (c) numbers of articles between 2014 and 2024 with the terms: "nano", "delivery", "atrazine" in the Web of Science.

Lignin is a natural organic macromolecule that came from lignocellulosic material and recently used in the fabrication of agricultural nanotools (Lima et al., 2021; Ahmad et al., 2021). Lignin is an enormous phenolic complex presented in the vegetal cell-walls, constituted by benzene units in its aromatic part and hydroxyl, carbonyls and ether functional groups in the aliphatic part (Figure 1A-1). This arrangement allows the formation of spherical, capsules and triangular shapes (Figure 1A-2), being spherical lignin nanoparticles (SLNPs) known due to their smooth surfaces (Österberg et al., 2020; Stanisz et al., 2022; Anushikha e Gaikwad, 2024; Behera et al., 2024; Dghoughi et al., 2025).

Lignin nanoparticles can be stocked without degradation for long time periods (Rodrigues et al., 2024), and can carry bioactive due to their ecological properties (biocompatibility and biodegradability) and good entrapment of active ingredients (Lima et al., 2021; Forini et al., 2022). For the nanoherbicides, lignin as matrix of nanocapsules (by nanoemulsion) and composite nanocapsules associated with polycaprolactone were presented to affect weeds (Restu et al., 2023; Restu et al., 2024; Takeshita et al., 2025). SLNPs are generally prepared by antisolvent (with water) method where the inner part is attributed to π-π benzenic rings and the surface is full of hydrogen bonds belonging to carboxylic-hydroxyl groups, in contrast to water lignin can form a nanosphere with hydrophobic core and hydrophilic shell (Trevisan et al., 2020; Santo Pereira et al., 2022).

For more efficient systems, herbicides such as atrazine (ATZ) can be carried out in possible ways: entrapping and adsorption for SLNPs and can be slow released in plants (Figure-1B) (Chandra et al., 2021; Song et al., 2024; Bryant et al., 2024). Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) is the chloride derivative herbicide most used in grass and broadleaf weeds – 90000 tons per year. (Rostami et al., 2021). Atrazine is a persistent organic contaminant with a half-time Ambiental cycle around 2 to 57 weeks (Ganjedra et al., 2024), leading to adverse effects in non-target plants: growth inhibition, chlorophyll reduction and photosynthesis, and oxidative stress (Jiang et al., 2023a).

Atrazine has been banned in the European Union since 2003 due to its soil and water contamination and bioaccumulation that causes negative effects in non-target organisms and human health (Wu et al., 2021; Pereira et al., 2014; Queiroz et al., 2024). From the 90,000 tons of atrazine used in the field per year, around 35,000 tons are from the United States of America (Zhang et al., 2025).

In Brazil, atrazine is the fourth most sold pesticide and can be found at a concentration of 3.3 μg/L in freshwater in the southern region (Brovini et al., 2023). Currently, some nanocarriers containing atrazine have been developed with promising results in literature. From 2014 to 2024, a total of 22 articles were found when the terms “nano”, “delivery” and “atrazine” were searched in the Web of Science (Figure 1C). Therefore, understanding how nanoherbicides can affect non-target crops and weeds (target) is essential for risk assessment in agriculture (Grillo et al., 2021, Chen et al., 2024).

In this thesis, different nanocarriers for herbicide delivery consisting of lignocellulosic materials were discussed in Chapter I. Subsequently, in Chapter II it was emphasized the development (preparation and characterization) of lignin nanospheres and their ability to carry the herbicide atrazine. In addition, the effect of this nanoherbicide was evaluated on weeds of the *Tridax procumbens* (coatbuttons) in soil and Lactuca sativa (lettuce) plants (non-target) in a hydroponic system. In Chapter III, the final conclusions of the study were presented highlighting its impacts and scientific-social relevance for agribusiness.

CHAPTER I - **Recent advances on lignocellulosic-based nanopesticides for agricultural applications**

Published article: Frontiers in Nanotechnology (Impact Factor 4.1)

Lima, P. H. C. D., Antunes, D. R., Forini, M. M. D. L., Pontes, M. D. S., Mattos, B. D., and Grillo, R. (2021). Recent advances on lignocellulosic-based nanopesticides for agricultural applications. *Frontiers in Nanotechnology*, *3*, 809329.

ABSTRACT

Controlled release systems of agrochemicals have been developed in recent years. However, the design of intelligent nanocarriers that can be manufactured with renewable and low-cost materials is still a challenge for agricultural applications. Lignocellulosic building blocks (cellulose, lignin, and hemicellulose) are ideal candidates to manufacture ecofriendly nanocarriers given their low-cost, abundancy and sustainability. Complexity and heterogeneity of biopolymers has posed challenges in the development of nanocarriers; however, nowadays the engineering toolbox for biopolymer modification has increased remarkably, which enables better control over their properties and tuned interactions with cargoes and plant tissues. In this minireview, we explore the recent advances toward lignocellulosic-based nanocarriers for the controlled release of agrochemicals. Also, we offer a critical discussion regarding the future challenges of this potential bio-based nanocarrier for sustainable agricultural development.

Keywords: nanopesticides, nano-enabled, sustainable agriculture, circular bioeconomy.

INTRODUCTION

The development of environmentally friendly tools that can produce crops or livestock without negative impact on humans and the environment is central to the Sustainable Development Goals. Nowadays, with the nanotechnology growth, researchers are getting good outcomes using nanomaterials (NMs) (Guo et al., 2021; Mattos et al., 2017; Sikder et al., 2021). For instance, engineered nanoparticles (ENPs) are capable of improving the efficiency of pesticides and fertilizers through the controlled release of agrochemicals, as well as by providing enhanced plant performance or to enable plants to act as real time as sensors, actuators, or electronic devices (Baker et al., 2017; Saleem and Zaidi, 2020; Agathokleous et al., 2019; Acharya and Pal, 2020; Usman et al., 2020; Singh et al., 2021, Grillo, et al., 2021a). The increased surface area to volume ratio of the ENPs enables a higher control of interfacial interactions with a given cargo to act upon demand or specific stimuli, but also many other features. Besides, several factors as surface charge, particle size, composition, solubility, and manufacturing methods can be harnessed to control the interaction of ENPs with specific plants and organisms (Jogaiah et al., 2021), with the goal of developing targeted systems. Although most of the current nanopesticides or nanocarriers are developed from synthetic (e.g. polymers) building blocks, several systems have been developed using biopolymers as tools for a controlled release system of agrochemicals (Mattos et al., 2017). Lignocellulosic-based nanopesticides are biodegradable and offer an interesting platform to produce safe-by-design nanopesticides as they can be non-toxic (Chamundeeswari et al., 2019; Shrestha et al., 2021; Zhang et al., 2019; Bhattacharyya., 2020; Ur Rahim et al., 2021) (**Figure 1**). Moreover, lignocellulosic can be obtained from agriculture side streams, which is ideal to reduce costs during the production of nanope sticides, which are intended to be applied in large scale agricultural operations. This strategy also indicates a *from plant-to-plant* effort, ideal for a circular bioeconomy landscape.

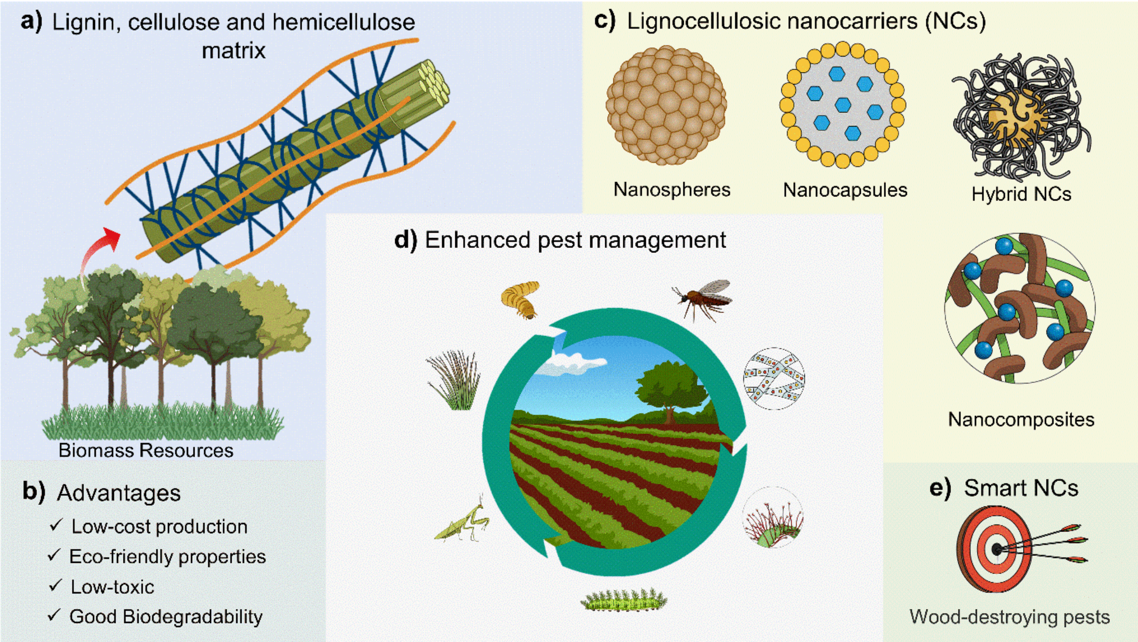


Figure 1. Schematic representation of **(a)** lignocellulosic building blocks matrix and their potential **(b)** advantages to produce **(c)** different types of nanopesticides, which can **(d)** enhance pest management (e.g. weeds, viruses, bacteria, fungi, and insects), as well as display unique properties such as **(e)** target to wood-destroying pests.

There are mainly three non-edible lignocellulosic biopolymers that have been used in the development of materials: cellulose, lignin, and hemicellulose, alongside pectin, tannins, acids, and proteins (Okolie et al., 2020). Even though the manipulation of its feedstock is challenging to engineer, lignocellulosic materials have low-cost production and eco-friendly properties (Li et al., 2021a). Although lignocellulosic-based nanocarriers are still growing for the agricultural sector, several advances have been performed to make this technology useful for nano-enabled agriculture. The increased use of lignocellulosics in nano-enabled agriculture is expected to take place because there are new technologies that can i) increase the extraction efficiency of lignocellulosic materials; ii) modify lignocellulosic structures for desired properties; and iii) design hybrid nanostructures with controllable shape and size. Nonetheless, the application of lignocellulosics in nano-enabled agriculture is currently related to the controlled delivery and release of pesticides and nutrients (Chen et al., 2020a; Teo and Wahab., 2020; Papadopoulos et al., 2019; Worrall et al., 2018). However, lignocellulosic nanomaterials could potentially stabilize emulsions (as those used in oil borne pesticides) instead conventional surfactants in multiphase systems

(Tardy et al., 2021). In this review will summarize and discuss the recent advances towards lignocellulosic nanocarriers for agriculture application, and also, we offer a critical discussion regarding the future challenges of lignocellulosic nano-enabled materials for sustainable agricultural development.

Types of lignocellulosic-based nanopesticides for agriculture

Cellulose-based nanopesticides

Cellulose is a linear polymer composed of several hundreds of glucose units, which are linked by β-1,4-glycosidic bonds (**Figure 2-a**). Cellulose is highly abundant, being sourced from agro-industrial biomass (from 1st generation to wastes and residues), marine biomasses, and microorganisms. (Kaya and Tabak., 2020; Teo and Wahab., 2020; Siqueira., 2020; Bahloul et al., 2021). Cellulose nanomaterials have been marketed in several sectors of the economy, including the agriculture. However, cellulose intra and intermolecular hydrogen bonding interactions hinders its dissolution in water, although being highly hydrophilic (Credou and Berthel., 2014; Shaktin and Kim., 2015). Therefore, several studies have focused on the chemical modification of cellulose using a variety of routes to modify its physicochemical properties, to enable dissolution and therefore to facilitate the conception of nanocarriers that can be applied for improving agrochemical efficacy (Rop et al., 2020; Machado et al., 2021). For instance, dialdehyde carboxymethyl cellulose (DCMC), and carboxymethyl cellulose (CMC) were conjugated with zein (a protein from corn) to develop nanocarriers for avermectin (Chen et al., 2020b; Hao., 2020a). In both cases, the resulting nanopesticides showed high leaf adhesion, as well as efficient protection of the active ingredient against ultraviolet light (UV) when compared to its conventional form. Avermectin has been also encapsulated in CMC grafted polyethyleneglycol (PEG) nanoparticles (Zhu et al., 2020) and in CMC grafted poly 2,2,3,4,4,4-hexafluorobutyl methacrylate (PHFBA) (Su et al., 2021a); both showing enhanced insecticidal activity against fall webworm (*Hyphantria cunea*) and an increase of the release profile of the active ingredient for 95h, respectively.

Additionally, glycine methyl ester (GLY) and glycidyl methacrylate (GMA) were used as intermediate and organic nitrogen sources, respectively, to modify CMC during the synthesis of a nanopesticide containing emamectin benzoate. Such nano pesticide-fertilizer increased the insecticidal activity against the diamondback moth insect (*Plutella xylostella*) as well as showed a potential system to be used as organic nitrogen fertilizer without toxic effect on seed germination (Zhao et al., 2021a).

On the other hand, nanoparticles crosslinked by cellulose have received an increase attention in the agricultural sector due to their good stability under environmental conditions (Sun et al., 2020). For instance, stimuli-responsive nanocapsules based on cellulose modified with fatty acid (undec-10-enoic) loaded captan and pyraclostrobin were developed against apple canker (*Neonectria ditissima*), and its results showed that the active ingredients were release triggered with the presence of cellulolytic fungi (Machado et al., 2021). Another study showed the fabrication of carboxymethylcellulose sodium salt and hydroxyethyl cellulose-based biodegradable hydrogels using citric acid (CA) as a crosslinker, and it was observed a good water uptake and a sustainable release profile (Das et al., 2021).

Mixture of (nano)cellulose with inorganic (nano)materials (for example clays) opens up strategies for the design of nanopesticides with a widened pallet of biological, thermal, and structural properties. For instance, carboxymethyl cellulose hydrogels filled with nanocellulose and nanoclays (Bauli et al., 2021) or cellulose-g-poly (ammonium acrylate-co-acrylic acid)/nano-hydroxyapatite (Rop et al., 2020) showed good efficiency on hydrogels nutrients' release as well as improve the moisture retention of the plant in the soil. Furthermore, a hybrid nanopesticide composed of hollow mesoporous silica/hydroxypropyl cellulose was reported to control rice blast fungus (*Magnaporthe oryzae),* and a dual-responsive release profile of the active ingredient was observed when in presence of cellulase (enzyme) or under acid conditions (Gao et al. 2021a). Moreover, layered double hydroxides (LDH) were mixed with CMC to fabricate polymeric nanocomposite able to mitigate the downside effect of herbicides in paddy cultivation (Sharif et al., 2021). Also, the observed high adhesion of nanocellulose towards hydrophilic surfaces has led to the development of biogenic nanohybrids containing biogenic silica and cellulose nanofibers for the encapsulation of various molecules including a biopesticide (thymol) (Mattos and Magalhães, 2016; Mattos et al., 2018).

The difficulty of promoting dissolution of cellulose, in parallel with the current regulation on bioplastics that defines cellulose derivative as plastics, will accelerate the development of nanocarriers from cellulose colloids, such as cellulose nanofibers, which have unique physicochemical properties such as higher surface area, nanodimension, high-temperature resistance, and biocompatibility (Shahi et al., 2021; Tardy et al., 2021). Cellulose nanofibers (CNFs) have been reported as potential high performers in nanoformulations in fertilization (do Nascimento et al., 2021), as well as cellulose nanocrystals (CNC) with chitosan to control tomato bacterial speck disease (Schiavi et al., 2021), among others (Table 1). For its diversity and abundance, cellulose is an outstanding material with a length of properties yet to be explored as a nanopesticide carrier, thus, understanding better its composition and potential interactions with agrochemicals and non- and target organisms are essential to the future design of sustainable nano-enabled agriculture.

Lignin-based nanopesticides

Lignin is a three-dimensional and complex aromatic biopolymer that is bound to cellulose and hemicellulose within the plant cell wall microstructure (**Figure 2-a**). Furthermore, it has attracted great attention due to its availability at large scales (Lizundia et al., 2021). In the last years, lignin-based nanoparticles (LNPs) have been used in agriculture against fungus, and insects due to their eco-friendly properties, low cost, and good encapsulating properties; however, it is insoluble in the aqueous environment, which raises some technical difficulties when producing nanocarriers but also to promote cargo delivery. Nevertheless, production of LNPs has been demonstrated to be passible of upscaling, and reasonable costs (Abbati et al., 2018; Ashok et al., 2018). For instance, stem lignin nanoparticles have been used as matrix in controlled release of the herbicide diuron (Yearla and Padmasree,2016). However, chemical modifications in lignin, to control their interactions with water and solubility (Balakshin and

Capanema 2015; Agustin et al., 2019; Ma et al., 2020; Machado et al., 2020; Falsini et al., 2019), facilitate their use in nano-enabled strategies for agriculture. For instance, sodium lignosulfonate can electrostatically interact with other polymers (e.g., chitosan (Li et al., 2019)), cationic surfactant (e.g. dodecyl dimethyl benzyl ammonium chloride (Zhang et al., 2021a), cetyltrimethylammonium bromide (Peng et al., 2020) by self-assembly to form stimuli-responsive nanopesticides (**Table 1**). Kraft lignin (KL) has also a tunable amphiphilic nature due to the abundant phenolic hydroxyl groups capable of forming a stable double-layered nanomaterial with ionic surfactants (Ela et al., 2021), nanocapsules with olive oil (Falsini et al., 2020), as well as being able to chelate cationic metals (Sipponen et al.,2016). Other nanopesticides have also been synthesized with alkali lignin (AL) (Yin et al., 2020), organosolv lignin (Zhang et al., 2020a), and methacrylate lignin (Yiamsawas et al., 2021).

In this regard, some interesting studies have been using lignin nanoparticles as a controlled release of fungicide to target Esca (a type of grapevine trunk disease that negatively impacts grape yields and the wine industry around the world). For instance, Machado et al., 2020 developed several fungicide-loaded lignin nanocarriers (**Figure 2-b_1, b_2)** to be applied in a single injection into *Vitis vinifera* ("Portugieser") plants, and the results showed successfully inhibition of the lignase-producing fungi (e.g. *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*), as well as fungicide efficiency for at least 4 years against Esca **(Figure 2-b_3)**. Furthermore, two other stimuli-responsive lignin-based nanocarriers were developed for the treatment of Esca (Peil et al., 2020, Fischer et al., 2020). In both studies, the fungi associated with Esca degraded lignin through secretion of ligninolytic enzymes (e.g., laccases and peroxidases), and thus released fungal spores *(Trichoderma reesei)* (Peil et al., 2020) and the hydrophobic fungicide pyraclostrobin (Fischer et al., 2020) loaded in lignin nanocarriers (**Figure 2-b_4,b_5**). Moreover, another triggered strategy is based on the controlled release of some metallic ions, such as copper and iron, to fertilize and protect plants against various pathogens (Guzzurelli et al., 2020; Li et al., 2021b).

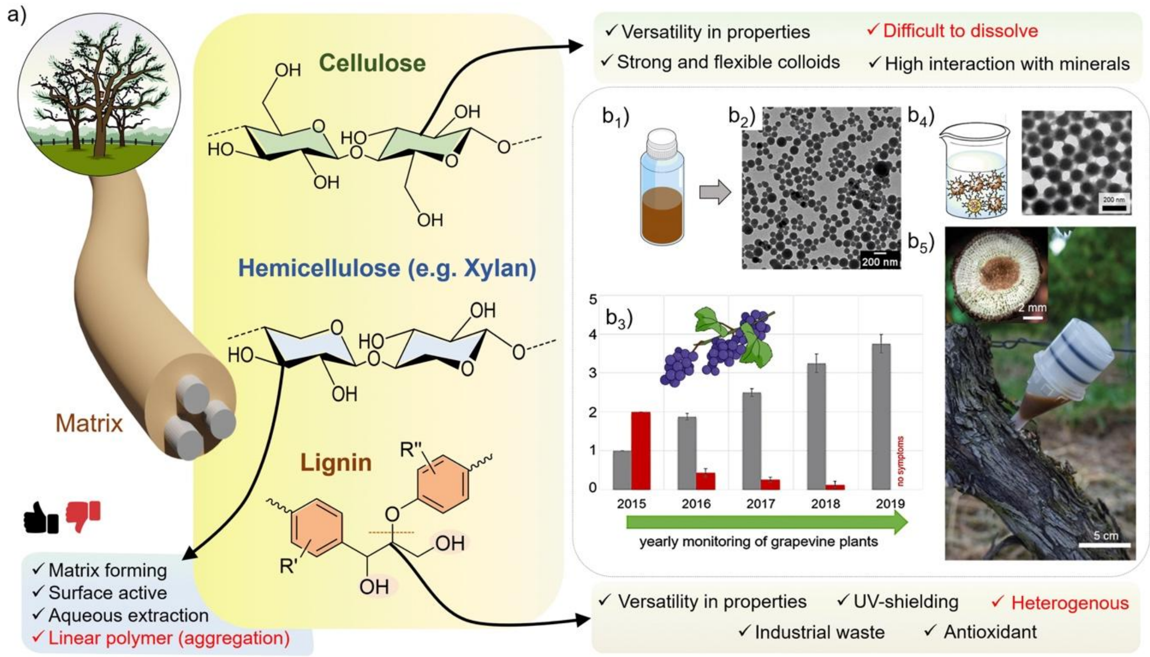


Figure 2. a) Types of lignocellulosic materials pointing out their main advantages (black) and disadvantages (red), **b_1)** representation of a formulation containing lignin nanoparticles, **b_2)** Transmission electron microscopy image of pyraclostrobin-loaded lignin nanoparticles in the range of 100-300 nm. **b_3)** Effect of empty lignin (gray bars) and fungicide-loaded lignin (red bars) NPs in Esca leaf symptoms on four Portugieser grapevine plants monitored yearly from 2015 until 2019, **b_4)** Transmission electron microscopy image of fungicide-loaded lignin NPs and **b_5)** Lignin based fungicide-loaded NPs being applied in a grapevine plant to target Esca. **b_2)** and **b_3)** were reproduced with permission from Machado, 2020 Copyright (2020) American Chemical Society, and **b_4**) and **b_5**) were reproduced with permission from Fischer et al., 2020 Copyright (2019) John Wiley and Sons.

Despite the development of numerous lignin-based nanocarriers for agriculture, the great challenge of these nanoformulations is still the complexity and variability of chemical structure of this resource given the great batch-to-batch variations in the extraction process. In this context, studies such as those by Beckers et al. (2021) have been important since it is possible to synthesize lignin-like monomers (e.g. phenylcoumaran and β-O-4-aryl ether) able to comprise linkages found in native lignin with promising results. Nevertheless, lignin-first biorefining approaches have recently been proposed to produce well defined lignin structures that can be further utilized in a more systematic way (Lourencon et al., 2019).

Hemicellulose-based nanopesticides

Hemicellulose is a biopolymer with degree of polymerization (ca. 50-200) and molecular weight below 90 kDa, thus a much smaller building block when compared to cellulose or lignin (Ye et al., 2021). Hemicelluloses represent 15-25% of the wood cell-wall but it can reach up to 45% in annual, seasonal plants. Such biopolymers include xyloglucans, xylans (**Figure 2-a**), mannans and glucomannans, and beta-glucans. A wide range of applications has been associated with hemicellulose, such as the generation of chemical products, packaging materials, drug delivery systems, and more recently as pesticide delivery systems (Wijaya et al., 2021; Naidu et al., 2018). The extraction of hemicellulose comes from lignocellulosic biomass, wood, foliage, grass, and agricultural residues and can be carried out using organic solvent but more commonly with hydrothermal extractions (Naidu et al., 2018).

Due to the low water solubility of hemicelluloses, there are still some challenges to produce stable hemicellulose-based nanocarriers for the controlled release of agrochemical. However, several examples have been demonstrated in the recent years. Beckers et al. (2020a) described the first synthesis of nanocarriers built from xylan extracted from corn cobs, to containing the fungicide pyraclostrobin, by interfacial polymerization method of diisocyanate of toluene (TDI) in an inverse emulsion. Hence, such nanopesticide was colloidally stable in water and cyclohexane for several weeks as well as it was efficient against phytopathogenic fungi (*Botrytis cinerea*) as the biocide release from the xylan nanocarriers was stimulated by the fungi. In another study, xylan-based nanoparticles (without active ingredients) were fabricated, and their antifungal effect was studied on corn husk fiber and on the high-density polyethylene (HDPE) composite. Such nanoparticles prevented the formation of hyphae in the wood as well as increased the strength of the composite (Gao et al., 2021b). Additionally, lignin sulfonate-based nanocarriers containing hemicellulose residues showed potential for controlled delivery of agrochemicals, such as the fungicides pyraclostrobin or prothioconazole (Beckers et al., 2020b). In addition, xylan-based nanoparticles were advantageous for their biocompatibility, biodegradability, and low-cost biological material (Beckers et al., 2020b). Recently, Lignin-xylan and arabinoxylan nanoparticles were also reported as an enzymatic responsive for pesticides release (Jiang et al., 2020) (**Table 1),** as well as a gene delivery system for CRISR-Cas9 DNA (Sarker et al., 2020), respectively.

β-Glucan is a homopolymer, an abundant class of polysaccharides in plants, fungi, and bacteria. Most recently, ß-Glucan based nanocarriers have been extensively studied as drug delivery systems (Su et al., 2021b). For instance, Kazien et al. (2022) developed a smart-delivery formulation based on carboxymethylated-β-glucans on the mesoporous silica nanoparticles (MSNs) surface after loading chlorothalonil (CHT) fungicide, with bioactivity against phytopathogens better than commercial formulation, and lower toxicity to manure worm (*Eisenia fetida)* and zebra fish (*Danio rerio*). In another study with the same formulation, the authors observed that CHT@MSNs-β-glucans showed 2.6 times lower toxicity to planktonic crustacean (*Daphnia magna)* and also exhibited lower effects on soil microbial abundance than commercial chlorothalonil (Kazien et al. 2021), which improves its use as a nano-enabled agrochemical.

Other lignocellulosic materials-based nanopesticides

Other lignocellulosic materials, even in minor quantities, are rising in agriculture as compounds of nanocarriers. Pectin (Pec), a structural compound present in the primary cell walls of higher plants has been applied for the development of nano-enabled delivery systems for agrochemicals. Pectin has been used to configure an intelligent stimuli-responsive carrier triggered by pectinase – enzymes produced by plants, filamentous fungi, bacteria, and yeasts. Moreover, a hybrid system comprising mesoporous silica nanoparticles and pectin (MSN-Pec) could delivery prochloraz (a fungicide) slowly, showing potential to be used in rice crops (Abdelrahman et al., 2021). Pectin-based nanocarriers showed promising behavior to mitigate drought stress in plants of arid and semi-arid environments (Sharma et al., 2017), and carbendazim-loaded chitosan-pectin nanoparticles showed a good response against pathogenic fungus *Fusarium pxysporum* and *Aspergillus parasiticus* (Kumar et al., 2017). Composite systems, such as chitosan/tripolyphosphate/pectin nanoparticles, were reported as a delivery system for paraquat herbicide to reduce the toxic behavior to alveolar and mouth cell lines, as well as to enhance the herbicidal activity against maize and mustard plants (Rashidipour et al., 2019). Thus, the nanoencapsulation of paraquat improves its herbicidal activity and reduces their toxic and mutagenic effects (Grillo et al., 2015; Pontes et al., 2021; Rashidipour et al., 2021).

Tannins, especially tannic acid, have been used in nanopesticide formulation (**Table 1**) as an additive to promote better foliage adhesion of particulate (Yu et al., 2019; Zhi et al., 2020). The ability of tannins to promote multiple and diverse secondary interactions towards virtually any surface has warranted their utilization to modify nanocarriers surfaces while adding UV protection and antioxidant properties (both useful to increase the lifetime of the active ingredient) (Guo et al., 2016). Finally, acids such as rosin (abietic acid) and salicylic acid are used in the attempt to create efficient nanocarriers (**Table 1**) (Su et al., 2021c; Zhao et al., 2021b).

Lignocellulosic-Based Materials	Composition	Size (nm)	Agrochemical	Findings	Ref
Cellulose	Sodium carboxymethyl celulose grafted by styrene/ methyl methacrylate and butyl acrylate.	180-280	Avermectin	The anti-ultraviolet photolysis ability of the active ingredient was improved and the release time was prolonged.	Chen et al., 2018
Cellulose	Myrtenal-Based Nanocellulose/ Diacylhydrazine	-	Boscalid and chlorothalonil	The complexes exhibited comparable or better antifungal activity than the commercial one against several fungi.	Li et al., 2021c

Lignin	Sodium lignosulfonate /Poly (vinyl alcohol)-valine	~ 10	Emamectin benzoate	Higher bioactivity of nanogels compared to non-nanoformulations.	Zhang et al., 2021b
Lignin	Sodium lignosulfonate	162	Pyraclostrobin cyclohexanone	Nanocapsules improved control efficacy on tomato crown and root rot compared to micro and other nanoformulations.	Luo et al., 2021
Lignin	Sodium lignosulfonate /Epoxy resin	150	Abamectin	Higher efficiency to control root-knot nematodes (*Meloidogyne incognita*) compared to conventional agrochemicals.	Zhang et al., 2020b
Lignin	Sodium lignosulfonate	150−250	Emamectin benzoate	Nanopesticide improved the photostability of the active ingredient, as well enhanced the insecticidal activity against *Prodenia. Litura* (leafworm) when compared to the commercial formulation. Also, nanopesticide exhibited pH-mediated release property.	Cui et al., 2019.
Xylan	Lignin/xylan	166 – 210	Avermectin	Lignin–xylan hybrid nanospheres showed well-defined core-shell structures with encapsulation efficiency above 57%. Also, nanopesticide showed enzyme-mediated release property.	Jiang et al., 2020
Tannic Acid	Fe^{3+}/Tannic acid	141-160	Tebuconazole	Nanocapsules showed high fungicidal activities against rice sheath blight (*Rhizoctonia solani*) and wheat head blight (*Fusarium graminearum*) pathogenic fungi.	Dong et al., 2021b
Tannic Acid	Mesoporous silica nanoparticles/	137	Pyraclostrobin		Liang et al., 2021

	Copper/Tannic acid			The complexes coating could improve the photostability of the active ingredient, as well enhanced deposition efficiency on rice leaves with good antifungal activity against *Rhizoctonia solani.*	
Abietic acid	Carboxymethyl celulose/ glycidyl methacrylate/ rosin	167	Avermectin	Nanopesticide displays enhanced dispersibility and stability of the active ingredient, as well as improves the affinity and light stability on cucumber leaf, maintaining good insecticidal activity against *Plutella xylostella*.	Zhao et al., 2021b
Salicylic acid	p-amino salicylic acid-modified polysuccinimide	155 - 290	Avermectin	Aqueous nanopesticides showed a cumulative release rate of 70 % with a pH-responsive profile. Also, it was reported good insecticide activity against *Plutella xylostella.*	Su et al., 2021c

Table 1. Examples of Lignocellulosic-Based Nanopesticides.

Concluding Remarks and Challenges Ahead

Current research on the smart nano-enabled delivery systems for pesticides has opened a new way to view the stimuli-responsive controlled release of crop protectants and to the development of novel agro-technological products. Despite the complexity of biomolecules and the challenges on generating stable nanoformulations, lignocellulosic-based nanocarriers have shown promising features that can be further explored in advanced, and renewable nanocarriers for agriculture. These carriers display unique properties (e.g. target to wood-destroying pests), low cost, and good biodegradability rate. However, a limited number of studies on the mechanism of action of these nanopesticides as well as their toxicity impacts in the environment is still very limited (Grillo et al., 2021b). Moreover, little is known about the biodegradation of biopolymers after their modification or compositing, which still restricts their wider utilization in large scale platforms such as crop protection. Therefore, further researchers on lignocellulosic nanomaterials are necessary in order to improve the efficient use of biomass resources as well as achieving environmental sustainability in agriculture.

Author Contributions

P.H.C.L, D.R.A and M.M.L.F. writing, review and editing the manuscript, M.S.P. and B.D.M. review and editing the manuscript, and R.G. conceptualization, writing, review, editing and supervision the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

The authors acknowledge funding from National Council for Scientific and Technological Development (Grant #427498/2018-0), São Paulo State Research Foundation (FAPESP), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil, CAPES – Finance Code 001. M.M.L. Forini thanks FAPESP for scholarship (#2020/12769-0).

Conflict of Interest

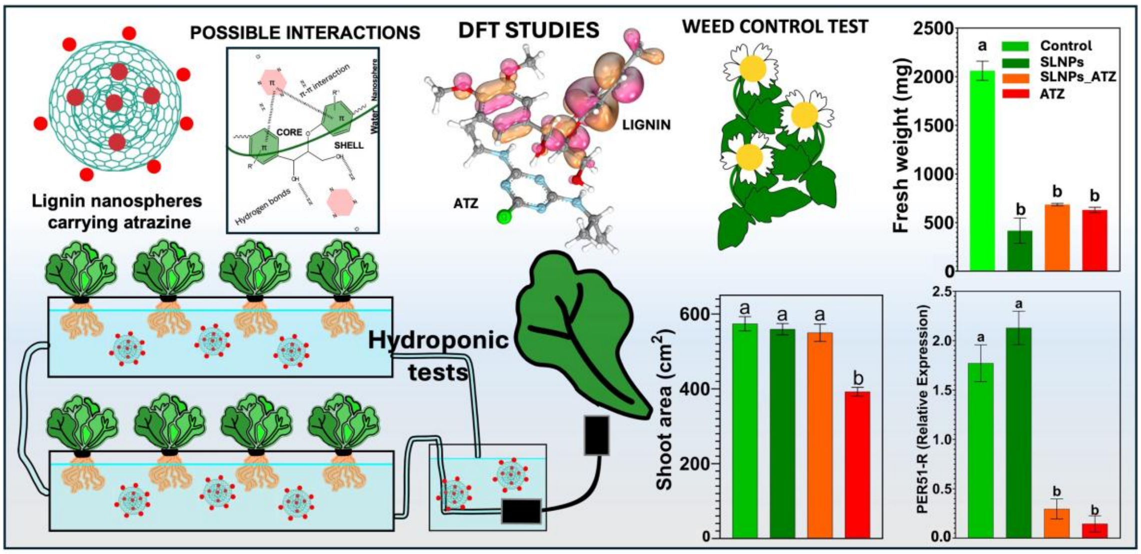
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CHAPTER II ***- Sustainable lignin nanospheres as atrazine carriers: preparation, characterization and evaluation of effects on target and non-target plants***

Sumitted to Sustainable Chemistry & Engineering (Impact Factor: 7.1)

Lima, P. H. C.; Buri, M. C. S.; Mendonça, R. S.; Favara, G. M.; Bischaldin, E. R.; Forini, M. M. L. H.; Cavalcanti, L. A. F.; Grillo, R.* *Sustainable lignin nanospheres as atrazine carriers: preparation, characterization and evaluation of effects on target and non-target plants. 2025*

GRAPHICAL ABSTRACT



Abstract

Weeds are known as undesirable plants, and farmers have been facing them through herbicides to enhance crop yields. However, herbicides also harm non-target plants and humans. In recent years, nanoscience research has reduced herbicide usage without losing crop quality. In this context, lignin emerges as a suitable candidate. Founded in plants and an agricultural waste residual, the lignin has a peculiar structure capable of forming sustainable nanospheres by an antisolvent precipitation method that may interact with herbicides (fabricating nanoherbicides) and slowly release them. In our work, spherical lignin nanoparticles (SLNPs) with atrazine (SLNPs_ATZ) were designed, characterized, and applied in non-target and target plants. SLNPs displayed a spherical shape with a size distribution of 161 nm when measured by DLS and 145 nm by NTA. SLNPs_ATZ also exhibited a spherical morphology, with a size distribution of 178 nm by DLS and 136 nm by NTA. The encapsulation efficiency was 74.2% and 43.26% of ATZ was released after 168 hours. Chemical computational modeling revealed lower gap energies between atrazine and lignin, indicating a strong carrier/bioactive interaction and corroborating with the release profile. Secondly, hydroponic experiments took place with butterhead lettuces (*Lactuca sativa var. capitata*) as model plants; a sublethal dose of atrazine (30 µg/L) was applied to keep lettuces alive for 28 days. Lettuces treated with SLNPs and SLNPs_ATZ showed no significant changes in root length and

shoot area compared to control, while lettuces treated with atrazine did. By biochemical tests, lipid peroxidation, and catalase showed significant differences comparing lettuce treated with atrazine and all the other treatments, while guaiacol peroxidase had SLNPs values lower than atrazine. By gene expression, CAT1 and GST6 genes expressed higher in lettuces treated with SLNPs. However, lettuces treated with SLNPs presented no physiological changes compared to control, indicating a possible tolerance from lettuces. Moreover, PER51 genes presented a repression in plants treated with SLNPs_ATZ and ATZ compared to control. By weed control assessment, seeds/seedlings showed possible germination/development interferences by SLNPs_ATZ, indicating lignin as a sustainable molecule for nanosphere construction and carrying atrazine with possible results in gene expression and control of weeds.
Keywords: lignin, nanoherbicides, atrazine, sustainable agriculture, non-target and target plants.

1. Introduction

The constantly growing world population causes nations to struggle with food shortages while farmers face crop turbulence with weed competition (Debaeke et al., 2024; Ghosh et al., 2024). Weeds compete with crops (Darbyshire et al., 2024) and have been engaged in a cycle of resistance and confrontation with chemical compounds in the last sixty-five years. Herbicides are chemicals used to protect plants against weeds; however, they are easily driven through leaching, drifting, or runoff into the water and accidentally harming off-targeted plant species, animals, and humans. Moreover, weeds have developed exponential resistance to herbicides, highlighting the need for new techniques to be developed and implemented (Perotti et al., 2020; Yu et al., 2021). Over time, agro-nanotechnology and herbicides have been used together to entrap and efficiently deliver bioactive to increase productivity and decrease the environmental impact (Fraceto et al., 2016; Grillo et al., 2016; Forini et al., 2022). Some nanoherbicides can delay the process degradation of herbicides (Cheng et al., 2024), improve the herbicidal activity (Pontes, et al., 2021, Oliveira, et al., 2015) and reduce the cytotoxicity to non-target organisms (Jin et al., 2025) and human cells (Dong et al., 2021a). Nanoherbicides can be derived from organic, inorganic, or hybrid materials (Forini et al., 2022), organic nanomaterials are quite formidable due to eco-friendly properties and effectiveness, they may minimize risks to non-target organisms and ecosystems (Kusumavathi, et al., 2024).

In this scenario, lignin is a natural organic macromolecule derived from agricultural waste. It comprises a complex phenolic structure in plant cell walls, formed by benzenes units in aromatic regions and hydroxyl, carboxyl, carbonyl, and ether functional groups in aliphatic parts. This arrangement allows the formation of diverse structures (e.g., capsules, triangular, and spherical), being spherical lignin nanoparticles (SLNPs) well-known for their smooth surfaces (Österberg et al., 2020; Stanisz et al., 2022; Anushikha and Gaikwad, 2024; Behera et al., 2024; Dghoughi et al., 2025). SLNPs are usually prepared by antisolvent precipitation method where aggregation forms nanospheres with hollows attributing to π-π interaction in the benzene rings and shells formed by hydrogens bonding among carboxylic-phenolic groups in water, forming hydrophobic core/hydrophilic shell structures (Trevisan et al., 2020; Santo Pereira et al., 2022). Recently, lignin has been explored to fabricate nanodevices in the agricultural sector (Lima et al., 2021; Ahmad et al., 2021). For instance, lignin based-nanoparticles can be stored for extended periods (Rodrigues et al., 2024), possess UV-blocking properties that may prevent active ingredients from degradation (Ma et al., 2025) as well can promote a controlled release of active ingredients (Lima et al., 2021; Forini et al., 2022). Also, lignin and lignin-composited nanocapsules (fabricated by nanoemulsion) were presented to have some efficiency in control *weeds* (Restu et al., 2023; Restu et al., 2024; Takeshita et al., 2025)

Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) is the most used chlorinated derivative herbicide in grassy and broadleaf weeds with 90,000 tons per year. However, it is a persistent organic contaminant with an environmental half-life ranging from 2 to 57 weeks (Ganjedra et al., 2024). It can also lead to adverse effects on plants, such as growth inhibition, chlorophyll and photosynthesis reduction, and the induction of oxidative stress (Jiang et al., 2023a). Atrazine has been banned in the European Union (but not in countries such as the United States and Brazil) since 2003 because its contamination and bioaccumulation affect soil and water, causing side effects on non-targeted organisms and human health (Wu et al., 2021; Pereira et al., 2024; Queiroz et al., 2024). Some studies show that nanoencapsulated atrazine can improve herbicidal activity (Grillo et al., 2016; Cordon et al., 2022), reduce toxicity (Forini et al., 2020), and enhance stability of the herbicide (Zhai et al., 2020).

In this context, lignin nanocarriers for the herbicide atrazine are still scarce in the literature, so lignin nanoparticles containing atrazine to study its herbicidal properties and its effects on non-target plants were developed. Previous studies showed that the interaction through π-π bonds with aromatic compounds and hydrogen bonds with hydroxyls are the reason for the atrazine removal in the aquatic environment (Akpinar et al., 2019; Hellal et al., 2024a). Thus, SLNPs may load the bioactive through encapsulation and adsorption (Wang et al., 2019), englobing ATZ due to its π-π and adsorbing the atrazine through hydrogen bonds-providing a strong interaction (Chandra et al., 2021; Song et al., 2024; Bryant et al., 2024).

On the other hand, understanding how nanoherbicides interact differently with plantas (Grillo et al., 2021a) non-target organisms (Zhai et al., 2020; Fojtová et al., 2019; Eghbalinejad et al., 2024) is essential to understand the risk assessment of agricultural products (Grillo et al., 2021b). Indoor hydroponics is an outstanding technique for assessing nanoherbicides with less external interference. Hydroponics has emerged as one of the most popular segments in modern controlled environment agriculture (Lei and Engeseth, 2021), favoring overall productivity by the manipulation of minimum space and water (Chowdhury et al., 2024), and leafy vegetables with short cultivation time such as lettuce draw attention (Sharifan et al., 2019; Majid et al., 2021). Lettuce (*Lactuca sativa L.*) is one of the most vegetables consumed worldwide. It is easy to cultivate and is a model plant because of its sensitive response when facing contaminants (Xu et al., 2018; Lian et al., 2020; Li et al., 2024). It has a higher ascorbic acid and polyphenols concentration and may prevent cardiovascular diseases (Shatilov et al., 2019; Xu et al., 2022). Proteins, polysaccharides, and lignin are also structures present in lettuce. The phenylpropanoid metabolism pathway creates Lignin and may provide stable and rigid bases for cell-wall structure, regulating the growth of roots and leaves (Shu et al., 2020; Yao et al., 2021; Xie et al., 2024). Hitherto, only one study correlates atrazine, nanoparticles, and lettuce in soil (Wu et al., 2021). For instance, 20μg/L of atrazine impacts *Allium cepa* (de Souza et al., 2023) in the hydroponic system, and it is also enough to cause phytotoxicity in macrophyte *Typha latifolia* (cattail) after 21 days (Perez et al., 2022). However, few studies have considered the aquatic environment as the fate of nanoherbicides in weeds (Preisler et al., 2022; Sousa et al., 2024), which can be essential to endorse new finds in agro-nanotechnology.

Here, lignin nanospheres and their ability to carry atrazine herbicide were studied in a hydroponic non-target plant species (lettuce) and target: *Tridax procumbens* - coat buttons in soil believing that our work will increase the knowledge of new nanocarriers in modern agriculture. The conduction of this research took place in three stages: i- preparation and characterization of SLNPs_ATZ, ii- Impacts on non-target organisms in the hydroponic system, and iii- Effects on target organism in the soil.

2. Materials and methods

2.1. Chemicals

Alkali lignin and atrazine were purchased by Sigma-Aldrich. Cloridic acid (HCL), methanol, *L*-methionine were purchased by Synth. Thiobarbituric acid (TCA), riboflavin, and hydrogen peroxide (H_2O_2) were purchased by êxodo. Starch was obtained by Dinâmica, and flex fertilizers were obtained from Plantapar. Potasium bromide (KBr). For RT-qPCR, cetyltrimethylammonium bromide was purchased by Sigma. Primers were acquired by Exxtend Biotecnologia, DNAse I- RNAse Free, and Oligo DT_{12-18} primer were obtained by Thermo Fischer Scientific, and SYBR Green PCR Master Mix by Promega. *Lactuca sativa var. capitata* seedings were purchased in the domestic market. Other reagents used in laboratory preparations and procedures were of a high degree of analytical purity. Milli-Q water with a resistivity of 18.2 MΩ.cm was obtained using a Direct-Q3 UV Smart system. Weed seeds (*Tridax procumbens)* were harvested in the local campus (Ilha Solteira).

2.2. Preparation of SLNPs and SLNPs_ATZ

Lignin nanospheres as carriers were prepared following Dai et al., 2017 method with modifications. Briefly, 27.5 mg of alkali lignin was dissolved and magnetic stirred in 5 mL of methanol for two hours. Later, 50 mL of water was added drop-by-drop (Figure 1a). Finally, the organic solvent was removed by rota evaporation until 10 mL of a colloidal solution remained in only water. In parallel, another solution of SLNPs with ATZ was prepared by adding 10 mg of ATZ with alkali lignin (final proportion 2.75:1 AL:ATZ [w/w]) in methanol and following the same previous procedure. The final concentration of ATZ in the nanoformulation was 1 mg/mL.

2.3 Physicochemical characterizations of the nanoparticles

Ultraviolet-visible (UV–Vis) spectroscopy (Shimadzu spectrophotometer - Model UV-1800) was used to study the absorption bands of SLNPs, SLNPs_ATZ, and ATZ with one part of formulation:64 of water dilution. The spectra were collected from 200 to 800nm.

The nanoparticles' hydrodynamic diameter, zeta potential, and polydispersity index were observed by Dynamic Light Scattering (DLS) in a dilution of 200 times (10:1990 µL) (Malvern Instruments Ltd., Malvern, U.K.). Also, analyses were performed to check the stability of the particles: on the first day after synthesis, seven days after synthesis, and multiple measurements until the seventieth day.

Nanoparticles tracking analysis (NTA) was performed by NanoSight Pro (Malvern Instruments Ltd., Malvern, U.K.) with a laser of 632 nm, dilution of 200 times (10:1990 µL).

Thermogravimetric analysis (TGA) was conducted using a TA Instruments SDT Q600 analyzer to evaluate the thermal stability of the nanoparticles. The test was performed under a synthetic air atmosphere, from room temperature to 1000 °C, at a rate of 10 $°C.min^{-1}$. Differential Scanning Calorimetry (DSC) using DSC – 25 analyzer, from room temperature to 200ºC, at a rate of 10ºC/min.

Scanning Electron Microscopy with coupled energy dispersive spectroscopy (SEM/EDS) was used to observe the morphology and size distribution of NPs. All the samples with a 200 times dilution (10:1990 µL) were measured using scanning electron microscopy (SEM, EVO-LS-15, Carls Zeiss), operated at 20kV with a spot size of 3.0–4.0 mm and a working distance (WD) of 9.5 mm.

The compounds (alkali lignin, NPs, and ATZ) were evaluated by Fourier transform infrared spectroscopy (FTIR, Nicolet 5DXB FTIR) in the 4000 to 400 cm^{-1} range, using 128 scans per sample with a resolution of 4 cm^{-1} using tablets of potassium bromide (KBr). All the analyses are more detailed in Text S1.

The physicochemical stability of the nanoformulations was established by hydrogen potential (pH) using a previously calibrated pHmeter (Tecnal). All the data were plotted as in GraphPad Prism 8.0 software.

2.4. Encapsulation efficiency and in vitro kinetics delivery assessment

The amount of herbicide associated with nanocarriers (encapsulation efficiency) was evaluated by the ultrafiltration/centrifugation method, which consists of centrifuging the nanocarrier suspension in ultrafiltration tubes composed of cellulose (Microcon, Millipore). Aliquots (300 µl) were filtrated by centrifugation (3000 rpm) for 10 minutes, then 100 µl were collected and diluted in 29.9 mL of deionized water (to fit Lambert-Beer law) and quantified by UV-visible. Encapsulation efficiency (%EE) followed eq.1 where (wf) is the final weight and (wi) is the initial weight.

$$\%EE = \frac{wf}{wi} \times 100 \qquad \text{eq.1}$$

To assess the kinetic release profile of SLNPs_ATZ, a two-compartment model (donor-receptor) was employed, where they were separated by a cellulose acetate membrane (Spectrapore®, with a molecular exclusion pore size of 1kDa). 500µL of SLNPs_ATZ solution were placed in the donor compartment (49,5mL of water). The system was gently stirred, and samples were taken over time (168 hours) from the donor compartment. Atrazine (222 nm) peak was analyzed using UV–visible spectroscopy. Additionally, a calibration curve previously validated of ATZ was used to quantify the release percentage.

The release data were fitted (M_0 /$M_{100\%}$ < 0.6) to the semiempirical Korsmeyer−Peppas kinetic model (eq. 2).

$$\frac{M_0}{M_{100\%}} = Kt^n \qquad \text{eq.2}$$

where M_O is the mass of atrazine released in interval t, $M_{100\%}$ corresponds to the total atrazine mass released at infinite time (100%), K is the release rate constant involving the structure and geometry of the system, t is time, M_0 is the total of atrazine in the time t, and n is the exponent indicative of the herbicide release mechanism, valid for Fickian and non-Fickian diffusion processes (Ritger and Peppas, 1987).

2.5. DFT studies and chemical reactivity

Density Functional Theory (DFT) studies were made by pre-optimized atrazine and lignin molecular structures, finding the most stable conformers by the Avogadro 1.2.0 program through random sampling with molecular mechanics techniques and a consideration of all the torsional angles. Since the lignin structure is huge, two model compounds were selected: 2-phenethyl phenyl ether (PPE) and bibenzyl (BB) (Yamaguchi et al., 2017).

The structure of the resulting conformer was then reoptimized and obtained on ORCA 5.0.3 program with the B3LYP\G functional and the def2-TZVP basis set. Water was used as the solvent, the images and the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) orbital energies (in eV) were obtained in IBOview, and electronic chemical potential (μ), chemical hardness (η), chemical softness (S) and electrophilicity index (ω) were calculated with LUMO and HOMO results, calculated according to Belakhdar et al (2020).

2.6 Effect of non-target organism

2.6.1 Lettuce in hydroponic treatments and morphological assessments

Sixty-four butterhead lettuce (*Lactuca sativa var. capitata*) seedlings were acclimated per one week in fertilized water at 16-26 ºC (a controlled environment) in a hydroponic system. The hydroponic system was set by two types of fertilizers in water, blue:red - 1:1.048 (w/w), compounds of the fertilizers are in Table S1, S2. Then, sixteen seedlings for each treatment (Control, SLNPs, SLNPs_ATZ and ATZ) were parted. After 7 days, 27 mL (from a solution stock – 10 mg/L) of ATZ solution were added to the tank, followed by fertilized water until a 9L point was reached, resulting in a 30 μg/L concentration. Then, 270 μL SLNPS_ATZ solutions (atrazine concentration: 1 mg/mL) were placed in their respective tanks, the same amount (270 μL) were used of SLNPs, and control was kept only in fertilized water. The length and area of the lettuce' shoot and root were measured in 0, 14, and 28 days. The width and area of leaves were measured on day 28. All measures were made by ImageJ through photos of the lettuce/leaves.

2.6.2. Chlorophyll and carotenoid contents

Chlorophyll a, b, and total were measured by a non-destructive assay, enabling continuous observation. The lettuces were analyzed in 0, 14, and 28 days with a digital chlorophyll content meter (clorofiLOG – FALKER) and measured in falker chlorophyll index (FCI). Carotenoids were calculated according to Ng et al., 2023. Using equations (eq. 3, eq. 4, eq. 5). After 28 days, 100 mg of lettuce leaves were macerated with 6mL of pure methanol, then the solution was centrifugated (3000 rpm, 10 min), and 2 mL were analyzed at spectrophotometer.

$$C_a\left(\frac{\mu g}{mL}\right) = 16.72\ A_{665} - 9.16\ A_{652} \quad \text{eq. 3}$$

$$C_b\left(\frac{\mu g}{mL}\right) = 34.09\ A_{652} - 9.16\ A_{665} \quad \text{eq. 4}$$

$$C_{(x+c)}\left(\frac{\mu g}{mL}\right) = (\ 1000\ A_{470} - 1.63\ C_a - 9.16\ C_b)/221 \quad \text{eq. 5}$$

2.6.3. Starch content levels

Starch concentration levels (mg/mL) were calculated after 28 days according to Rawashdeh et al (2020) and Antunes et al (2024) with modifications. Lettuce samples (1g) were soaked into sodium phosphate buffer and incubated at 37 ºC for 10 minutes. Next, a solution of soluble starch (1%, w/v) was added, mixed, and incubated at 37 ºC for 15 min. HCl was added to stop the reaction, followed by an iodine reagent (iodine potassium [KI] and iodine [I_2]). Absorbance was measured at 620 nm using the spectrophotometer, and a standard calibration curve previously validated by Antunes et al. (2024) with five different concentrations was used to measure the final concentration.

2.6.4. Lipid peroxidation

Malondialdehyde (MDA) levels content was inspired by Wang et al. (2023) and Behtash et al. (2024) methods. After 28 days, lettuce roots and leaves (1 g) were crushed using a mortar and pestle in 5 mL of 10% trichloroacetic acid. After 3 minutes of centrifugation, 2 mL of the supernatant was mixed with 2 mL of 0.67% thiobarbituric acid; the reaction mixture was placed in a bath for 30 min at

95ºC, ice-cooled and centrifuged for 3 min. The absorbance of the supernatant was measured at 450, 532, and 600 nm. MDA content was calculated using eq. 6 and eq. 7, where w is the weight of samples.

$$MDA\ concentration\ \left(\frac{\mu mol}{L}\right) = 6.45\ (A_{532} - A_{600}) - 0.56\ A_{450} \qquad \text{eq. 6}$$

$$MDA\ content\ \left(\frac{\mu mol}{g\ FW}\right) = MDA\ concentration\ X\ 5\ /m \qquad \text{eq. 7}$$

In addition, other aldehydes (1-hexanal, 1–butanal, 1-propanal, and 1-propanaldehyde methyl acetal) were measured according to Fotovvat et al. (2024), by the calculation of absorption of 455 nm using the extinction coefficient of 0.457 X 10^{-5}M cm^{-1}.

2.6.5. Peroxidase, catalase, and superoxide dismutase activity

The enzyme extraction followed Azevedo et al (1998) with modifications where 2.5 g of leaves and root (after 28 days) were macerated with 10 mL of refrigerated extraction formed by 3 mM of Dithiothreitol (DTT) and 1mM ethylenediaminetetra-acetic acid (EDTA) in a phosphate buffer (pH 7.0) with 4% (w/v) of polyvinylpyrrolidone. Then, the homogenate was centrifuged at 10000 rpm, 4ºC for 20 min.

Guaiacol peroxidase (POD) activity was measured by the addition of 90 µL of the extract, 2 mL phosphate buffer (pH 7.0), 200 µL of 1% (v/v) guaiacol, and 100 µL of 0.16% (v/v) H_2O_2 with absorbance measured at 470 nm by spectrophotometry after one minute. The POD activity was calculated using the molar extinction coefficient of 2.47 mM^{-1} cm^{-1}, and the enzyme activity was expressed as nmol H_2O_2 oxidized min^{-1} g^{-1} FW. The catalase (CAT) activity was determined using a reaction mixture containing 3 mL of 12.5 mM H_2O_2 in phosphate buffer (pH 7.0) and 100 µL of the extract. The decrease in absorbance was measured at 240 nm with a gap of two minutes, and CAT activity was calculated using the molar extinction coefficient of 39.4 mM^{-1} cm^{-1} and expressed as nmol H_2O_2 decomposed min^{-1} g^{-1} FW. The enzyme contents POD and CAT were determined according to Chance and Maehley (1955).

The superoxide dismutase (SOD) activity was determined according to da Silva Santos et al. (2020) with modifications. Briefly, 50 µL of extract were mixed with 2 mL of a bigger solution containing 9.6 mL of phosphate buffer (pH 7.0), 6 mL of Nitro Blue Tetrazolium (NBT) (0.44 mM), 6 mL of L-methionine (6.5 mM), 2.4 mL of 1 mM riboflavin, and 6 mL of EDTA (50 nM) - 30 mL in total, and exposed to fluorescent light (20W) at 25 ºC for 15 minutes, control was kept in the dark and the SOD activity was measured at 560 nm in the spectrophotometer, being SOD activity the difference between the absorbance of lighted and unlighted, the result was expressed in units of SOD (U) g^{-1} FW.

2.6.6. FTIR of lettuces

Briefly, after 28 days, samples of the four types (control, SLNPS, SLNPS_ATZ, and ATZ) of lettuce leaves and roots were put in the stove at 37 ºC per four days. Then, 10 µg of samples and 140 mg of KBr were measured, macerated, compressed, and analyzed in the FTIR. Finally, the second derivation was analyzed by plotting the data in GraphPad Prism 8.0.

2.6.7. Gene expression analysis

2.6.7.1. Total RNA Extraction

Six plants per treatment were randomly selected for leaf and root sampling for total RNA extraction. From each plant, 100 mg of root tissue was weighed, and 1.2 cm diameter discs were collected from three distinct leaf sections: the base, center, and margin. RNA extraction was performed according to Dolye and Dolye, 1987. In this method, leaf and root tissue was crushed in 400 µl of cetyltrimethylammonium bromide (CTAB) extraction buffer and incubated for 30 min at 65 ˚C. Subsequently, 400 µl of chloroform was added, followed by vortexing for 1 min and centrifugation at 13000 rpm for 5 min. The supernatant (approximately 200 µl) was transferred to a new microcentrifuge tube, and 200 µl of isopropanol was added, mixed, and kept at -20 ˚C for one hour. The samples were centrifuged at 13000 rpm for 5 min at 4 °C, the supernatant was discarded, and the pellet was washed with 200 µl of 70% ethanol at 13000 rpm for 5 min. The supernatant was discarded, and the pellet was dried. Finally, the pellet was resuspended in 50 µL of nuclease-free water, treated with DNAse, and the extracted RNA was stored in an ultra-freezer at -80 °C.

2.6.7.2. cDNA Synthesis and qPCR Gene Amplification

RT-qPCR technique was applied to identify oxidative stress gene expression levels using the real-time PCR system (BioRad, USA). The GAPDH, PER551, CAT1, and GST6 gene expression levels (Table 1) were measured using the GoTaq® 1-Step RT-qPCR System (Promega, USA). In a total volume of 10 µL, the reactions were performed using 2 µL (50 ng/µL) of RNA, 5 µL of GoTaq® qPCR Master Mix, 2X, 2,5 µL of ultrapure water, 0,1 µL of GoScript™RT (Promega, USA) and 0,2 µL of each primer pairs at a concentration of 10 µM. In thermocycler, the reactions were carried out at 37 °C for 30 min, the initial denaturation was held at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing and extension at 60 °C for 30 s. A final melting temperature step was set at 65 – 95°C for 0.5 s. Real-time PCR efficiencies were obtained using the LinRegPCR software version 2021.2. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene. The relative expression level was calculated using the $2^{-\Delta\Delta CT}$ method (Livak et al., 2001).

Table 1 – Primers selected for gene expression analysis

Name of gene	Primer sequences (5‘– 3‘)
CAT1	5’ – GGTCCAAGGCGATGTCTTTG – 3’
	5’ – ATGAACAGCTGGCGTTTTGT – 3’
PER51	5’ – CTGTCAACACATGGGCTTCC – 3’
	5’ – TCCCACTTCGACCCGTTTTA – 3’
GST6	5’ – GCCCAAATACTTGCTCTCCG – 3’
	5’ – TTGGGATGACTACCGACGAG – 3’
GAPDH	5’ – AGGTAGCGATCAACGGATTC – 3’
	5’ – AGGTGGGATGCTTGTTTGAC – 3’

2.7. Effect of target organism in sublethal doses, chlorophyll, and fresh weight

Ten seeds of *Tridax procumbens* were sowed in forty pots (n=8) in a greenhouse at mild condition (maximum temperature: 28ºC and three-time irrigation) per one day. Pre-emergence treatments with pure atrazine (4.2 g a.i

.ha^{-1}); SLNPs_ATZ in a solution of equivalent grams of pure atrazine; SLNPs (with the same quantity of SLNPs) and control (water) were conducted. Photos were taken on days 14 and 28. Fresh weight was measured after 28 days, and chlorophyll levels were measured in *Tridax procumbens* seedlings.

3. RESULTS AND DISCUSSION

3.1. Physicochemical characterizations of the nanoparticles

Lignin has a unique structure with hydroxyls and benzenes rings in abundance. The usage of this biomolecule to fabricate nanospheres may be entangled with the simple procedure of antisolvent precipitation. During the process, lignin dissolves in a solvent (methanol in our case) and forms spherical in contact with water (antisolvent), it is believed that the structure can self-assemble due to h-bonding (shaping the surface) and π-π stacking in the core. (Wang et al., 2024; Wang et al., 2025a). Through all four forms of carrying a bioactive by nanoparticles, two ways of how SLNPs can carry ATZ can be hypothesized in this work: surface and cavity loading (Wang et al., 2019). In our experiment, atrazine is mixed with alkali lignin in methanol and during this time two possible interactions (Figure 1A) may be observed: hydrogen bond between lignin hydroxyls and atrazine amines and π-π stacking among the aromatic structures. These schematic interactions are shown in other molecules with similar form to lignin (with benzene rings and hydroxyls) and ATZ, in their cases the organic compound was used to remove atrazine (Akpinar et al., 2019; Xing et al., 2020 Wang et al., 2025b). In our case, since atrazine was put together with lignin, it is possible that part of atrazine is carried inside the nanospheres' cavities and part is adsorbed by SLNPs surfaces while antisolvent precipitation is taken. Although, the mechanism needs to be studied in the future.

The integrity of spherical morphologies was observed and nanoparticles with good dispersion were observed in SEM by both: SLNPs and SLNPs_ATZ (Figure 1B-1; B-2). In addition, the relative percentage frequency distribution (%) of the nanospheres with and without atrazine were plotted and it was noticed that SLNPs (Figure S1-A) had most of their sizes located between 140 and 150 nm, this method with methanol presented smaller size compared to other solvents as tetrahydrofuran (THF) where spherical nanoparticles ranging 250 - 350 nm (Lee et al., 2021; Lu et al., 2021). Moreover, nanoparticles with atrazine - SLNPs_ATZ (Figure S1-B) had higher peaks around 110 - 120 nm. Posterior, DLS and NTA of SLNPs (Figure 1B-1) analysis were made, and by DLS the nanoparticles had around 160 nm (mean: 161.4 ± 1.93 nm). By NTA they presented similar results (with a mean of 145 ± 45 nm) where the partial percentages were 6.12% in the range of 20 - 80 nm, 87.28%: 81 - 205 nm and 5.87%: 206 - 300 nm, the SLNPs size around 145 ± 45 nm is near the one obtained by Dai et al (2017) where they found sizes of 131.2 nm. Moreover, SLNPs_ATZ (Figure 1B-2) showed a range around 154 – 203 nm (with a mean of 178.6 ± 24.7 nm) by DLS, although they were slight lower in NTA analyses (with a mean of 136 ± 43 nm): 1.57%: 251 – 350 nm, 25.61%: 151 – 250 nm and 72.1%: 60 – 150 nm.

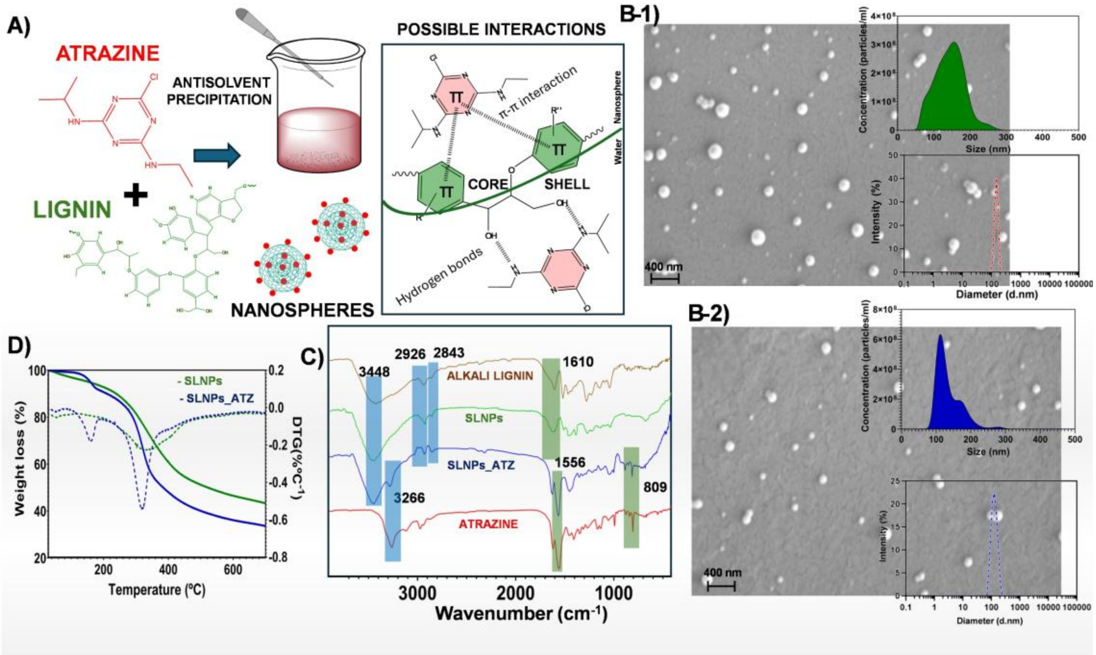


Figure 1 – (a) Preparation of spherical lignin nanoparticles with atrazine and possible interactions (b-1;2) SEM, DLS and NTA results of SLNPs and SLNPs_ATZ, (c) FTIR of Alkali lignin, SLNPs, SLNPs_ATZ and ATZ, (d) TGA analysis of of SLNPs and SLNPs_ATZ,

By the FTIR spectrum of pure atrazine (Figure 1C), N-H stretching at 3266 cm^{-1}, deformation of triazine group at 1556 cm^{-1}, and C-Cl stretching at 809 cm^{-1} were indicated. All the previous peaks match SLNPs_ATZ FTIR, suggesting the presence of pure atrazine outside of the nanospheres that may be correlated with the adsorption of herbicide by SLNPs (Peng et al., 2025). Hydroxyls of lignin phenolic groups are marked at 3448 cm^{-1} in SLNPs_ATZ, SLNPs, and alkali lignin (AL) (Zhang et al., 2022). Also, all ligno-structures presented bands located at 2926 and 2850 cm^{-1} provided from the symmetrical and asymmetrical C-H stretching of the methyl and methylene. Nonetheless, only AL and SLNPs showed bands around 1610 cm^{-1} related to aromatic skeletal vibrations of lignin (Freitas et al., 2020).

Also, a peak was observed at 275 nm by UV-Visible spectrometry analysis (Figure S-1C) that is characteristic of spherical lignin nanoparticles (Xiong et a., 2017), In addition, TGA analysis (Figure 1D) showed thermic differences between SLNPs and SLNPs_ATZ TGA curve of SLNPs_ATZ exhibited a variation around 190 ºC as compared to SLNPs, which can be correlated to the melting temperature (Tm) of free ATZ (around 175 ºC) – not encapsulated (Liu et al., 2024). This slight rise of Tm may be to possible strong hydrogen bond interaction between the lignin-atrazine. Also, TGA of lignin nanoparticles had similar behavior to the ones found in the literature. A rapid weight loss is readily observed under 100 ºC by SLNPs inferred to the water molecules captured by the hydrophilic groups of SLNPS. After, a rapid decline in weight loss between 200~450 ºC was correlated to inter-unit linkages such as aryl ether bond (such β-O-4) linkages that started to pyrolyze and may lead to lignin oxidation. Aromatic ring the strong C–C bonds were slowly decomposed above 500 ºC. Finally, char residue was observed at 700 ºC (Guo et al., 2022; Gong et al., 2025).

By DSC analysis (Figure S-2A), SLNPs glass transition (Tg) were noticed around 120ºC, in literature Tg of lignin can reach 160ºC (Sharma et al., 2022). In Figure S-2b, the Tm of ATZ in DSC corroborated with TGA curve. For example, Gautam et al. (2025) showed only a peak of ATZ around 181ºC and correlated to Tm of the compound. Since SLNP_ATZ presented another peak around 95ºC, this can indicate a displacement of SLNPs peak (decrease of Tg) which may confirm the theorization of previous paragraphs. Part of the herbicide is outside of the nanoparticles (and interacting by hydrogen bond - adsorbed) and part is loaded. This behavior - Tg decreasing values of nanoparticles when loading of herbicide - were observed in PLA nanoparticles and metribuzin (MTZ) (Stloukal et al., 2012; Shakiba et al., 2020).

Subsequently, more formulations were done to do stability tests over time, and nanospheres without/with atrazine showed sizes (Figure S-3A) of 178.96 ± 60.88/268.20 ± 69.58 nm on the first day of analyses by DLS with Zeta Potential of -26.43 ± 1.36 / -30.83 ± 2.45 mV (Figure S-3B) and a polydispersity Index (PdI) (Figure S-3C) of 0.44 ± 0.16/0.46 ± 0.16. In the last day (seventh), nanospheres (with and without atrazine) presented sizes around 151.80 ± 5,79 / 208.50 ± 7.68 nm, zeta potential of -31.70 ± 2.76/ -22.61 ± 0.94 mV and a PdI of 0.29 ± 0.08/ 0.29 ± 0.08. For knowledge, nanoparticles with zeta potential above ± 30 mv/ ± 20 mv present good electrostatic/steric stabilizations (Kamble et al., 2022), stability of nanoparticles over time is important for storage and our lignin nanoparticles present a great stability after 70 days, this may be cause of the electrostatic repulsion forces on their surface among hydroxyl groups and π-π stacking interactions (Moreno and Sipponen, 2024). The pH stability of nanospheres is also important, solutions with fitting the range between 3 and 9 present stable formats. Our formulations presented pH of 6.22 - SLNPs and 6.51 - SLNPs_ATZ on the first day, and 6.83 and 6.48 on the seventieth. In fact, over time pHs had no extreme variation (Figure S-3D) and remained around 6 ~ 7: (Moreno and Sipponen, 2024).

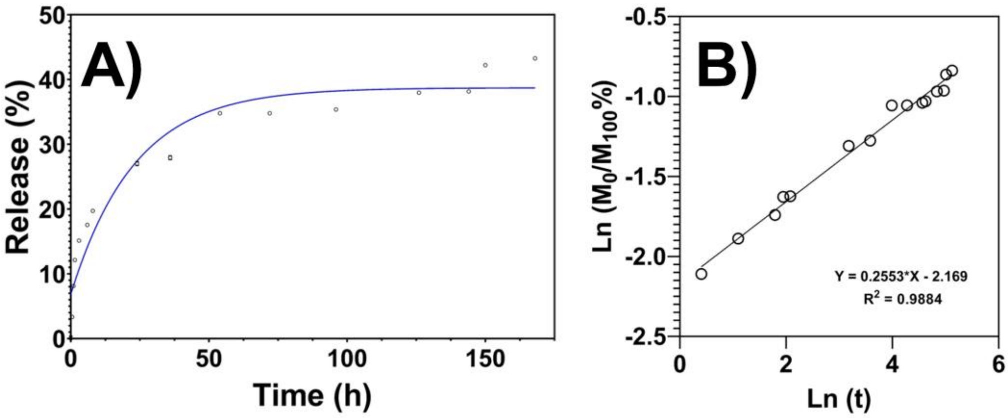


Figure 2 – (a) Release curve of spherical lignin nanoparticles containing atrazine (b) Release data fitted to the Korsmeyer-Peppas kinetic model.

Furthermore, the loading efficiency was studied and a 74.2% in a 2.75:1 AL:ATZ (w/w) was found, suggesting an interaction between lignin nanospheres and the chemical. For comparison, Dai et al (2017) reached loading efficiency of

71.2% while carrying resveratrol (dissolution 100 mg/L in water while atrazine has 33mg/L) in the proportion of 2:1 AL:resveratrol. Also, Carvalho et al. (2023) presented zein nanoparticles with polomaxer and polysorbate (as surfactants) with encapsulation efficiency above 90%. Moreover, PCL nanoparticles (nanocapsules) presented encapsulation efficiency above 84% of atrazine (Grillo et al., 2012). It is important to reinforce that most nanocarriers of atrazine have surfactants/oils. On the order hand, our formulation has only alkali lignin and water, reducing the amount of chemicals and the final cost of the product.

The kinetics release profile of herbicide carried by SLNPs_:ATZ was conducted by a two-compartment assay, as previously described by Grillo et al 2014 where only free ATZ passes through the cellulose membrane. Atrazine release (Figure 2A) reached 43.26 % in 168 hours. For comparison, Takeshita et al., 2025 prepared nanoparticles of polycaprolactone (PCL) carrying MTZ and PCL-lignin-MTZ and compared their release in the first 24 hours, PCL-MTZ reached 70% of release and PCL-lignin-MTZ presented 31.67%, indicating a higher interaction between lignin and bioactive once a similar result was obtained- reaching a 27% release in 24 hours and 43.26% after 168 hours, this indicates a strong lignin – atrazine interaction. Korsmeyer-Peppas (Figure 2B) was model that fitted the release profile where release rate was considerable high in the initial hours and tapered off over time. By the equation of Fig.2B the slope of the curve is diffusion index (n) is 0.2553, and n values less than 0.45 fit Fickian diffusion mechanism (Ge et al., 2021). Other kinect models are expressed in Table S3.

3.2. DFT studies and chemical reactivity

Understanding the interaction of organic materials by molecular modeling techniques such as DFT - computational modeling that allows the identification of essential points on the potential energy surface - has become crucial in exploring and reproducing at the laboratory (Houston et al., 2022; Sherard et al., 2023). In this work, DFT studies showed the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of atrazine and two model compounds: Bibenzyl (BB) and 2-phenethyl phenyl ether (PPE) - dimers models of lignin that have already been used in DFT studies (Yamaguchi et al., 2017). With LUMO and HOMO values it can be estimated the kinetic stability and chemical reactivity of the systems (Cheng et al., 2023a). Considering that only ATZ, lignin and water as solvent were involved, DFT studies may be extremely important to understand the interaction among the molecules.

By the LUMO-HOMO gap, the value of 5.19 eV for ATZ in water was (Figure 3A), and a 5.77 eV for ATZ without water in the iteration - matching the values of literature (5.7 - 6.0 eV) (Hellal et al., 2024a; Hellal et al., 2024b), although they did not consider the solvent water in their calculations. BB energy gap was around 5.3 eV and when it was put together (BB-ATZ) in water, the energy gap diminished to 3.75 eV. Normally, decrease of energy gap indicates better stability between two compounds (Liu et al., 2025). The same behavior occurred with PPE which energy gap jumped from 5.21 eV to 4.24 when interacting with ATZ (Figure S4). With LUMO and HOMO values, the chemical properties (Table 2) were calculated.

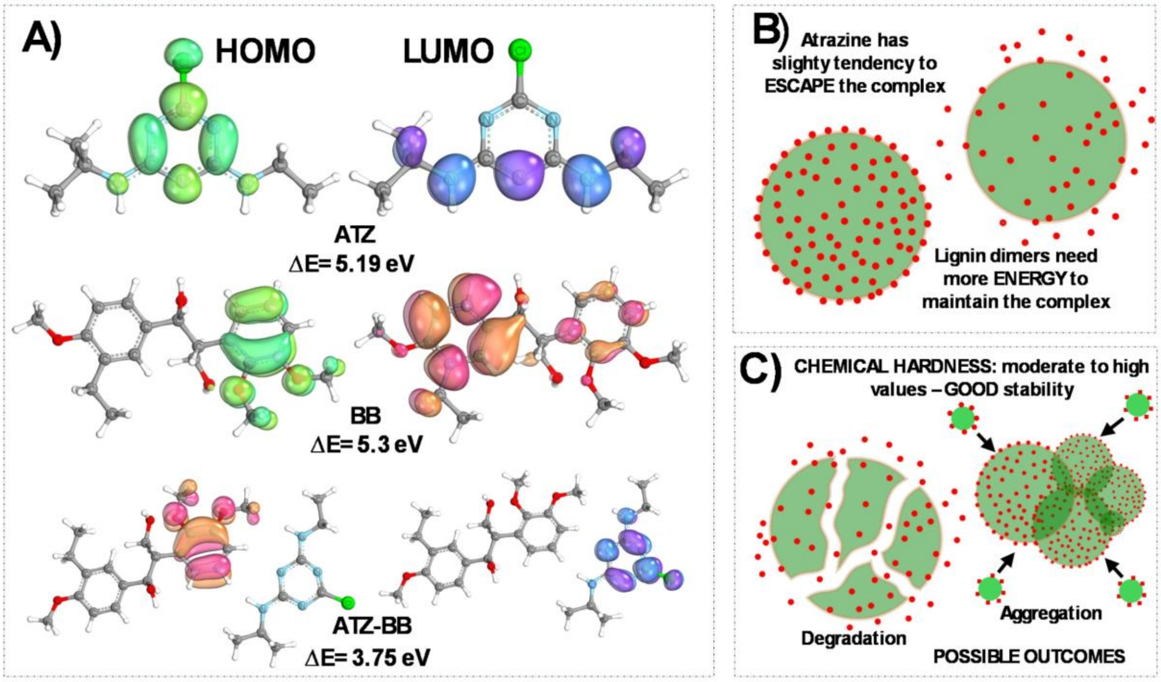


Figure 3 – (a) LUMO and HOMO (DFT results) of ATZ,Bibenzyl and the interaction of ATZ-bibenzyl, (b) scheme of release profile: energy to maintain the complex, and (c) stability evaluation the SLNPs_ATZ.

Recently, DFT studies have been complementing loading efficiencies and releasing mechanisms of nanosystems in experimental-theoretical combination investigations (Sathishkumar et al., 2021). Starting with electronic chemical potential (μ) – a parameter that indicates electrons tendency to escape from an organic complex, slightly higher results of BB-ATZ and PPE-ATZ compared to ATZ indicate a low tendency ATZ to leave the complex and (Figure 3B) possible slower release. The electrophilicity index (ω) is a property that quantifies the amount of energy required to maintain a molecule stable during electron transportation, lower ω means higher resistance to exchanging electrons with the environment. Our work found higher values for BB-ATZ and PPE-ATZ when compared to lignin dimers, may indicate more energy to maintain ATZ - lignin by the nanocarrier (Hoffmann et al., 2024).

The chemical hardness (η) measures the resistance of a molecule to change the number of electrons in the system, higher values of η means a more stable complex, and softness (*S*) is capacitance of the molecule to acquire charge (Pearson., 1993); Ben Haj Fraj et al., 2022). Our value of chemical hardness diminishes from ATZ: 2.95 eV; BB: 2.65 and PPE: 2.6 eV to ATZ-PPE:2.11 ATZ-BB: 1.875. Although, a value around 1.8 eV is moderate to high, meaning that the complex lignin-atrazine is stable after formation, avoiding (Figure 3C) possible rapid degradation or agregagtion (Mirza et al., 2025). In this work, the interaction from ATZ to lignin elucidated by DFT merges with its higher loading efficiency) and slow-release data (Bryant et al., 2024; Zhang et al., 2024a).

Table 2 – HOMO, LUMO, chemical potential (μ), chemical hardness (η), softness (S) and electrophilicity index (ω)

Structure	E_{HOMO}	E_{LUMO}	**μ**	**η**	**S**	Ω
	eV	Ev	eV	eV	eV^{-1}	eV
ATZ	-6.61	-1.42	-4.015	2.595	0.3854	4.03
BB	-5.63	-0.33	-2.98	2.65	0.3774	1.67
PPE	-5.61	-0.40	-3.00	2.6	0.3846	1.73
BB-ATZ	-5.62	-1.87	-3.745	1.875	0.5333	3.74
PPE-ATZ	-5.60	-1.36	-3.48	2.11	0.4739	2.87

3.3. Physiological and biochemical assessments

3.3.1. Physiological assessments of exposed lettuces

Recently, a work published by Parkison et al (2022) notified that organic (or soft) nanoparticles with negatively charged surfaces can gradually accumulate in the xylem of roots, putting our nanoparticles in a relevant position. Of course, all discussion about nanoherbicides and non-target species are in its initial phases (Hennig et al., 2023), subsequently, our research focus on negative charged and organic nanoparticles originated from plants (lignin) to evaluate hazardous effects in non-target plants (e.g.: lettuces) aims the enrichment of future knowledge in modern agro-nanotechnology.

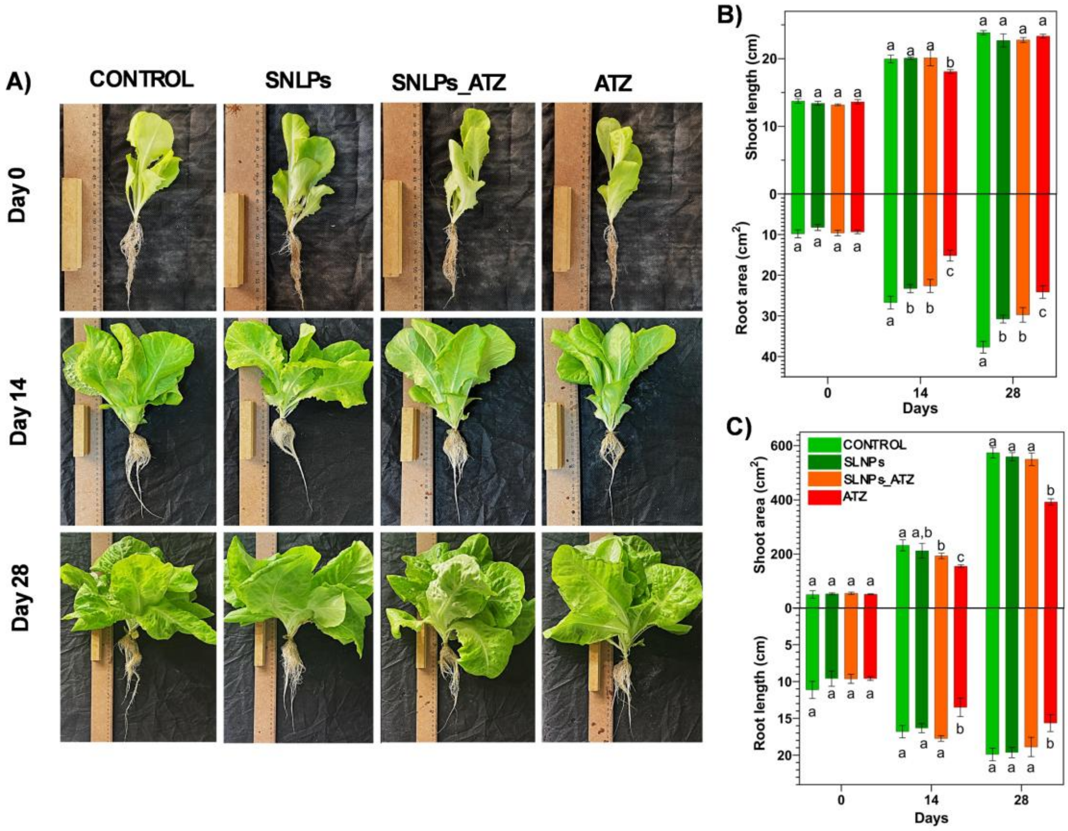


Figure 4 – (a) Photos of lettuce with the four treatments and at day 0, 14, 28, (b) lettuces root and shoot lengths and (c) shoot and root areas. SLNPs and ATZ containing at 30 μg/L of ATZ. Both formulations (SLNPs_ATZ and ATZ) contained 30 μg/L of ATZ. Statistics were made by two-way ANOVA and pairwise multiple comparison and tukey tests were used to analyze trueness (α=.0,05) in GraphPad Prism 8.0.

Many herbicides in soil/aquatic systems accumulate in certain organelles/tissues and can face plant detoxification mechanisms when absorbed by roots/shoots and modify plants morphology. Atrazine (in lethal doses) herbicide can surely attack non-target roots of some plants as lettuces, and their lengths may serve as important responsive bioindicators of pesticide damages (Zhang and Yang, 2021; Lescano et al., 2023; Queiroz et al., 2024; Shahid et al., 2024; Jiang et al., 2024). Here a ruler with a handle was put to compare lettuces (Figure 4A) on day 0, 14 and 28 with all treatments, and root length (Figure 4B) had significant change comparing Control; SLNPs; SLNPs_ATZ and ATZ (Day 0 – 11.1cm; 9.6 cm; 9.6cm; 9.6 cm to Day 28 – 19.9 cm; 19.6cm. 18.9 cm and 15.6 cm), presenting a difference of 27.56% between atrazine (30 μg/L) - control while SLNPs and SLNPs_ATZ presented no significant changes. In another study, treatment with ATZ statistically presented the most drastic difference compared to control, lettuce seedlings were reported to have a 33.1% decrease in root length with 10 μg/L of atrazine in 125 hours (Lescano et al., 2023). Moreover, shoot area (Figure 4C) increased with similar behavior as root length (% increasing of shoot area comparing day 0 and day 28: Control - 1027%; SLNPs - 947%; SLNPs_ATZ - 869% and ATZ - 654%).

Although root area (Figure 4C) had a significant change between control, SLNPs - SLNPs_ATZ, ATZ, being SLNPs and SLNPs_ATZ having the same significance (being higher than ATZ and lower than control), inferences in root's growth by nanoparticles is suggested, however, the increasing were comparable higher than ATZ (% increased at day 28: Control - 284%; SLNPs - 273%; SLNPs_ATZ - 210% and ATZ - 159%). Shoot length (Figure 4B) presented no significant changes over time. Nonetheless, leaves width and area (Figure S5-A, B) did, plants exposed to atrazine had 23.43% and 32.76% smaller width and area sizes when compared to the control. Moreover, the results of plants exposed to atrazine compared to SLNPs_ATZ and SLNPs had similar behavior (33% - 23% area - width smaller than plants with SLNPs_ATZ; 41.15% - 37.64% area - width smaller than plants with SLNPs), SLNPs did not interfere in the leaves sizes while interacting with non-target species as butterhead lettuce. Curiously, xenobiotic chemicals such as atrazine may cause various detrimental effects such as shoot deformation, and reduction in the leaf size but SLNPs_ATZ also did not cause leaf malfunction (Shahid et al., 2024).

3.3.3. Starch, chlorophyll, and carotenoids

Long-term starch storage provides resources for plants to recover after damage and assists plants in resisting external stresses (Ribeiro et al., 2022). After 28 days (Figure 5A), we observed a concentration of 0.656 mg/mL to control, 0.839 mg/mL to SLNPs, 0.797 mg/mL to SLNPs_ATZ and 0.992 mg/mL to ATZ in the lettuce's shoot where ATZ presented a statistically significant increase compared to nanoparticles and control. Additionally, nanoparticles presented intermediate behavior, notable increased value compared with control. Starch in root's lettuce had a slightly different behavior (Control, SLNPs, SLNPS_ATZ, ATZ: 0.626, 0.932, 0.943 1.273 mg/mL), where control and SLNPs presented a similar behavior, ATZ had a significant increase compared to control and SLNPs, and SLNPS_ATZ had an intermediate value. These findings have comparable results to Jiang et al., 2023b, where they state that atrazine deeply interferes with starch synthesis in soybean leaves (off-targeted plant), rushing starch accumulation and abetting atrazine stress resistance. By FTIR of leaves and roots (Figure S5- C, D), we identified a peak around 1000 cm^{-1} belonged to carbohydrates and indicated a correlation with starch accumulation, since starch is a carbohydrate and lettuce exposed to only atrazine presented a huge clear difference from nanoparticles and control (Wang et al., 2024).

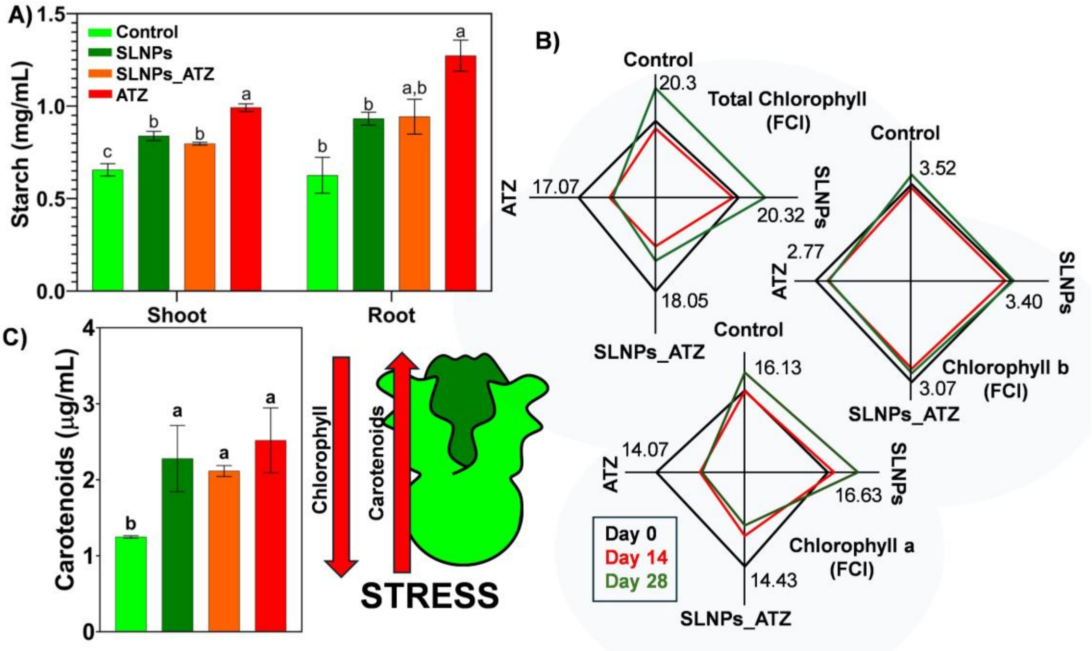


Figure 5 – (a) Starch content levels after 28 days, (b) total chlorophyll, chlorophyll a and b levels at day 0,14 and 28, and (c) Carotenoids after 28 days. Both formulations (SLNPs_ATZ and ATZ) contained 30 µg/L of ATZ. Statistics were made by two-way

ANOVA and pairwise multiple comparison and tukey tests were used to analyze trueness (α=.0,05) in GraphPad Prism 8.0.

Atrazine is a known-herbicide inhibitor of chlorophyll by retarding protoporphyrin IX formation - chlorophyll synthesis precursor (Vieira et al., 2021). Atrazine also influences enzymatic mechanisms like peroxidase, catalase, and carotenoids (Neto et al., 2024). Carotenoids influence photosynthesis and antioxidant activity and contribute to reactive oxygen species (ROS) removal (Del Buono et al., 2021). After 28 days, our study (Figure 5B) found an increase of total chlorophyll, chlorophyll a and b (Control: 7.82%, 4.19%, and 9.97%; SLNPs: 6.76%, 7.08%, and 1.87%) in plants with no treatment and treated with SLNPs, presenting no statistical difference between the two treatments. On the other hand, plants treated with SLNPs_ATZ and ATZ showed decreasing in all chlorophyll levels (Table S4) - SLNPs_ATZ (total chlorophyll: - 7.53%; chlorophyll a: - 9.39% and chlorophyll b: - 8.78%) and ATZ (total chlorophyll: - 8.81%; chlorophyll a: - 10.58% and chlorophyll b: - 13.45%).

SLNPs_ATZ presented no significant changes in chlorophyll b levels with SLNPs, however, a significant difference compared to ATZ was noticed. Also. ATZ and SLNPS_ ATZ had a significant gap compared to Control/SLNPs in total chlorophyll, indicating no chlorophyll synthesis perturbance from SLNPs. Carotenoids concentration (Figure 5C) in the control was 83.06% less than SLNPs, 73.06% less than SLNPs_ATZ, and 102% less than ATZ, control presented a significant difference compared to all the other treatments. Normally, carotenoids accumulate in plants under abiotic stress (as atrazine herbicide), and this work obtained comparable results (Swapnil et al., 2021).

3.3.4. Antioxidant enzymes (ROS)

Primary antioxidant enzymes are a class of proteins/metalloproteins that first react and rapidly catalyze reactive oxygen species into more stable ones. ROS grow in response to detrimental pesticidal effects on plants, counteracting their toxicity through metabolic processes in their many internal defense systems to detoxify or/and decompose these chemicals (Wang et al., 2022; Jomova et al., 2023; Shahid et al., 2024; Gohar et al., 2024). MDA is a ROS parameter that measures lipid peroxidation - rupture of the cell membranes. MDA from lettuce exposed by atrazine (Figure 6A) revealed no significant changes in the shoot, however, root MDA levels showed significant differences comparing ATZ with all the other treatments (40.51%, 69.83%, and 50.92% huger than Control, SLNPs, and SLNPs_ATZ) (Taban et al., 2020). For a possible nanoparticles comparison, 0.02 mg/mL concentration of lignin – graft - poly(lactic-co-glycolic) acid (PLGA) nanoparticles (zeta potential around - 53.6 mV) in a soybean germination hydroponic environment demonstrated no MDA significant changes when compared to control after 14 days, despite our concentration were 667 times (30 µg/L) lower than Salinas et al., 2021, we also found no significant changes between SLNPs and control in shoot. It is important to highlight that the monomers of PLGA - lactic acid and glycolic acid – participate in plants' life cycles (Harper et al., 2022; Zhao et al., 2024), and our concentration was very low because lettuce seedlings died within 8 days in a 300 µg/L of ATZ – a non-desirable effect to a longer time comparison.

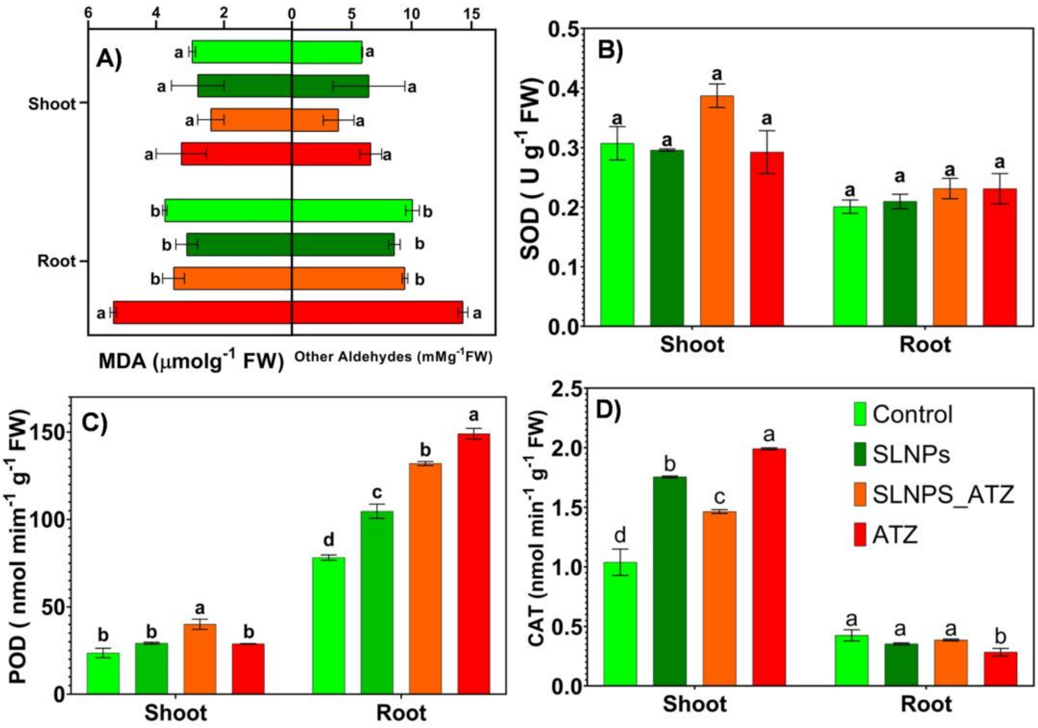


Figure 6 – ROS (a) MDA and other aldehydes, (b) CAT, (c) SOD and (d) POD. Both formulations (SLNPs_ATZ and ATZ) contained30 µg/L of ATZ. Statistics were made by two-way ANOVA and pairwise multiple comparison and tukey tests were used to analyze trueness (α=.0,05) in GraphPad Prism 8.0.

In another work, nanoparticles containing polycaprolactone – negative charge surface and xenobiotic material - and lignin carrying herbicide metribuzin (MTZ) were indicated to have a triple efficient in killing *Amaranthus retroflexus* than PCL-MTZ nanoparticles at 48 g a.i. ha^{-1} after 21 days of application while a less concentrated significant MDA content was notified by PCL-lignin-MTZ than PCL-MTZ nanoformulations at 480 g a.i. ha^{-1} in soybean (non-target) plants (Rodrigues et al., 2024; Takeshita et al., 2025). Furthermore, in our research, other aldehydes have similar results: atrazine had a bigger significant difference compared to other treatments in the root (41.18%, 66.76%, and 51.14% bigger than Control, SLNPs, and SLNPs_ATZ).

Xenobiotic/non-xenobiotic organic nanoparticles have effectively been tested in non-target plants to understand the limits and effects of their usage. For example, zein/poloxamer nanoparticles were recently reported to be good nanocarriers of glyphosate with no apparent damage in cotton/soy and show the effects on soil enzymes (Munhoz-Garcia et al., 2025). On the other hand, chitosan (xenobiotic) nanoparticles (positively charged surface) were used as paraquat (a non-selective herbicide) carriers and tested in spinach plants (leaves), Pontes et al (2021) notified a significant change CAT enzyme level concentration at a 20 μg/L when comparing the herbicide to the chitosan/chitosan paraquat nanoparticles, and chitosan nanoparticles presented significative CAT content changes compared to chitosan: paraquat nanoparticles/ paraquat at 30 μg/L exposal.

Catalase is one of the most crucial antioxidant enzymes occurring in aerobic organisms such as plants and englobes peroxidases classification types, CAT normally breaks down two molecules of H_2O_2 into 2:1 water: oxygen molecules (Zhao et al., 2021c; Jomova et al., 2023). In a work published by Takeshita et al (2025), significant statistical difference among control, MTZ, PCL-MTZ, and PCL-lignin-MTZ in CAT were noticed after 30 days in soybean plants exposure to particles. Our work (Figure 6B) presented significant changes in the lettuce treated with ATZ compared to the other treatments - with lower levels of CAT - at the root. However, shoot leaves showed significant changes comparing ATZ to control (91.71%), SLNPs (68.71%), and SLNPs_ATZ (40.65%) where SLNPs presented more significant levels of POD than SLNPs_ATZ, but lower CAT levels caused by SLNPs and SLNPs_ATZ are indicated.

SOD is another primary responsive enzyme that removes O_2 radicals in the phospholipid membranes (Alscher et al., 2002). SOD (Figure 6C) results showed no significant changes after 28 days in our work, although plants treated with SLNPs_ATZ and ATZ had apparent differences in shoot area. Moreover, guaiacol peroxidase (POD) is a glycoprotein from the cell wall and tangles with fundamental processes such as defense against abiotic/biotic stress and lignification of cell walls (Sobiecka et al., 2022). Guaiacol is quite used to evaluate peroxidase activity since it oxidized and turns into tetraguaiacol (which has the maximum absorbance at 470 nm) in the presence of hydrogen peroxide (Niu et al., 2021; De Oliveira et al., 2021). Our research found considerable results when comparing all treatments (Figure 6D). In the shoot (leaves) part, SLNPS_ATZ showed showed higher significant POD concentration. However, SLNPs had a statistical similarity with control and SLNPS_ATZ/ ATZ. Furthermore, a descending significant concentration is found in the roots part (ATZ> SLNPs_ATZ > SLNPs > Control).

3.3.4. Effect of treatments on defense genes (RT-qPCR)

Reverse-Transcription quantitative polymerase chain reaction (RT-qPCR) analysis was performed to quantify the effect of different treatments (control, SLNPs, SLNPs_ATZ and ATZ). For our analysis, GAPDH was selected as reference gene due to its better stability over others as actin, ubiquitin, and protein phosphatase (Chowdhury et al., 2015). PER51, CAT1, and GST6 genes were purchased by Chowdhury et al., 2019 where they used *Arabidopsis thaliana* as the genetic model organism for lettuce and confirmed all the sequences. Also, all these genes (PER51, CAT1, and GST6) were previously tested in abiotic stresses in treating lettuce (Bigott et al., 2021).

First, catalase1 (CAT1) gene, an antioxidant enzyme that is known for possible scavenge ROS, and protection of plant cells from oxidative damage was tested (Di et al., 2023), presented a big significant difference between control - SLNPs (increased 2164%) and atrazine (669%) in root (Figure 7B). Thus, the same behavior was identified in shoot (Figure 7A) part - SLNPs: 81.08%; ATZ 98.65%, both presented high values of gene expression although lettuces treated with SLNPs and SLNPs_ATZ had no significant physiological changes in shoot/root area and root length. It is important to mention that plants normally repress growth as an adaptive strategy to maximize survival, and lettuces treated with ATZ did that (Zhang et al., 2023).

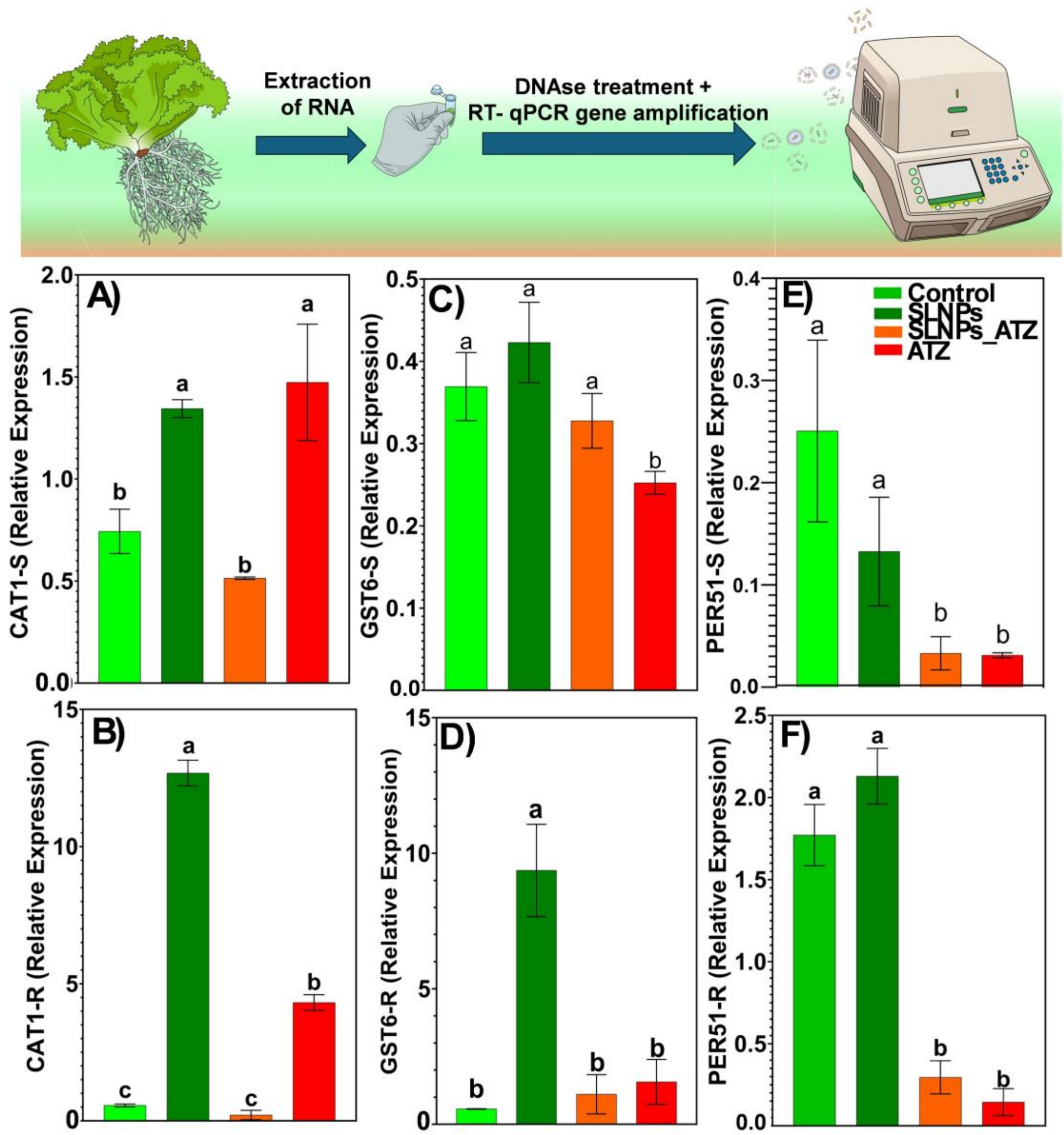


Figure 7 – Relative expression of (a b) CAT1, (c, d) GST6 and (e, f) PER51 genes. Both formulations (SLNPs_ATZ and ATZ) contained 30 µg/L of ATZ. Statistics were made by two-way ANOVA and pairwise multiple comparison and tukey tests were used to analyze trueness (α=.0,05) in GraphPad Prism 8.0.

Glutathione S-transferases (GSTs) are a class of enzymes that detoxify chemicals and secondary products of oxidative damage, GST6 genes expressed in response to auxin, SA and hydrogen peroxide (Chowdhury et al., 2019; Zhao et al., 2020). Increased GST expression levels protect organisms against oxidative stress (An et al., 2020), and a highly increased (1546%) level was observed by SLNPs treatment in root (Figure 7D) when compared to control, tolerance by lettuce is suggested since no significant enormous physiological changes (only in root area) was noticed. Inherently, SLNPS_ATZ did not have the same behavior - showing no significant changes, representing a possible effect of atrazine that may have downregulated the expression in root part since GST6 genes in the shoot part (treated with atrazine) presented a significant decrease (46,4%) when compared to control (Figure 7C).

PER51 genes (Figure 7E, F) participate in Class III peroxidases - responsible for some functions such as lignification, cell wall biosynthesis, ROS detoxification, and auxin pathway regulation (Simoni et al., 2024). Recently, PER51 were notified as LsPRX11 genes by Simoni et al., 2024, they indicated that *LsPRX11 (PER51)* genes are correlated with the regulation of PHR (phosphate starvation response) transcription during P starvation in lettuces (Zhou et al., 2021). Here they were less resistant in a 30 μg/L atrazine exposure, since SLNPs_ATZ and ATZ presented decreasing relative expressions compared to control at the (SLNPs_ATZ: 86.8%; ATZ: 87.6%) shoot and (SLNPs_ATZ: 83,3%; ATZ:91,9%) root, indicating a possible repression of these genes caused by atrazine (injuring the plants). Comparable effects were described by Ramel et al., 2009 where they observed repression in ascorbate enzymatic (a member of the heme-containing peroxidase family) activity and designated a consequent disruption of antioxidant mechanisms by atrazine injuries in *Arabidopsis thaliana* (a genetic model plant). Moreover, in our research, SLNPs presented a non-significant change compared to control, suggesting that PER51 genes were not affected by the spherical nanoparticles

It is crucial to highlight that catalase and peroxidase enzymatic activities do not match gene expression results in our studies. The catalase that was picked - encoding as LOC111878432- has already been used in some recent works with lettuce (Zhang et al., 2024b; Wang et al., 2025c) – but is only one of two catalase genes of lettuce (LOC111878431) (Kohai et al., 2020). Then, it should not be discarded the possibility that catalase activity is more correlated to CAT gene LOC111878431. However, our atrazine levels between enzymatic activity and gene expression in shoots had similar behavior. The same can be inferred from our peroxidase assays, the genes of guaiacol peroxidase (POD) and PER51 genes belong to peroxidases class III, although recently ninety-one peroxidases class III genes were published (Simoni et al., 2024).

The application of nanoparticles and the up-regulated expression of genes related to biotic and abiotic stress responses are not unusual (Tripathi et al., 2022), and the potential of nanoparticles in enhancing plant tolerance to pollutant stress is notified in literature. For example, dioxide titanium nanoparticles were recently notified as reliever of atrazine phytotoxicity effect to lettuce (Weng et al., 2025). However, the relative expression of genes is not well understood, mainly when the nanoparticles express specific genes in enormous values in treatments' comparison. For example, "cold-regulated 413" - COR143 genes play crucial pieces to plants stressed by cold. Recently, an article showed an up-regulated behavior in lettuce treated with carbon dots (near ten times higher than control) where the authors notified a cold tolerance caused by the nanoparticles (Cheng et al., 2023b). For comparison, the values presented here up-regulated around 16 times in GST6 genes when control and SLNPs in root were contrasted. In another study, bio-synthesized silver nanoparticles improved the growth of rice seedlings and had CAT and APX genes up-regulated by around 5 and 9 times when comparing rice treated with nanoparticles (10 mg/L) to control (Gupta et al., 2018).

3.4. Pre-emergent treatments of weeds in sublethal doses

Once 30 µg/L of ATZ in hydroponic experiments was used and all lettuce did not die, the same quantity of bioactive mass was tested for soil (4.2 g a.i. ha^{-1}) of ATZ in *Tridax procumbens* seeds in pre-emergent treatments, atrazine normally is recommended in doses around 850 g a.i. ha^{-1} of atrazine (mixtured with 1,65 kg i.a. ha^{-1} of metolachlor) for *Tridax procumbens* (Chikoye, D et al., 2005). In other seeds different effects were observed, as in Amaranthus retroflexus germination when exposed to several sublethal doses of atrazine (18.75 ~ 600 g a.i. ha^{-1}) showing important results such as less germination percentage between 1/32 and 1/16 of formulation compared to the recommended dose (1200 g a.i. ha^{-1}) (Qi et al., 2018). [153] Our research had a n= 8 (where n is the number of pots), and pots with control and atrazine treatments had clearly higher gemination rates in seeds (Figure 8) than the ones treated with SLNPs and SLNPs_ATZ.

Results of nanoparticles with herbicidal effects are seen in literature as positive signs to lower herbicide dosages/usages, and non-xenobiotic and xenobiotic nanoparticles (to plants) were already related to having higher efficiency in lower doses when compared to atrazine. For example, PCL (xenobiotic) nanocapsules with atrazine presented the same inhibitory effect in root and shoot growth of *Amaranthus viridis* and *Bidens pilosa* seedings with concentrations ten times lower than conventional (2000 g a.i. ha^{-1}) in Brazil (Sousa et al., 2018). Moreover, zein (non-xenobiotic) nanoparticles with atrazine had greater herbicidal activity against *Brassica juncea* at doses 80 times (25 g a.i. ha^{-1}) lower than the recommended on pre-emergent treatments (Carvalho et al., 2023).

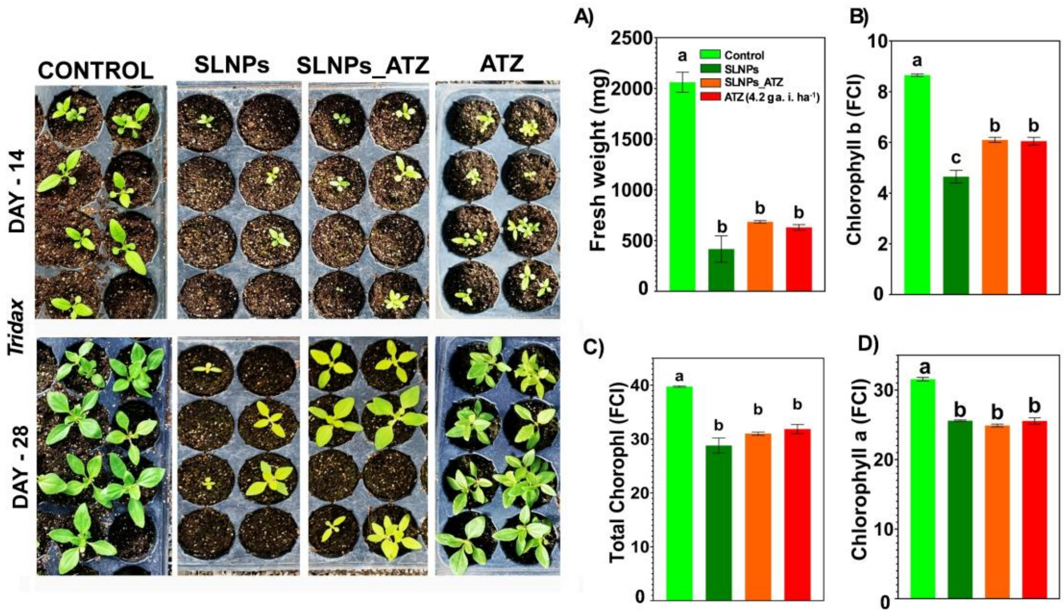


Figure 8 – Photos of *Tridax procumbens* after 14 and 28 days. And (a) Fresh weight of *Tridax procumbens* and (b,c,d) profile of *chlorophyll b,* total and a. Statistics were made by two-way ANOVA and pairwise multiple comparison and tukey tests were used to analyze trueness (α=.0,05) in GraphPad Prism 8.0.

Another parameter worthwhile to correlate is the material used in this work: lignin. It was already tested with PCL in nanoformulations for *Amaranthus retroflexus* in pre-emergent treatments by Takeshita et al.; 2025, where they notified a fresh mass 3.45 times lower from PCL-lignin-MTZ than PCL-MTZ in a

48 g a.i. ha^{-1}. They also highlighted the importance of lignin (non-xenobiotic) as a natural molecule that is more suitable for a safe-by-design herbicide nanoformulation. Moreover, Munhoz et al. (2025) presented zein-lignin nanocarriers synthesized with glyphosate - as the active - and indicated a reduction or interference of the nanoherbicides in weed plants (*Amaranthus hybridus, Ipomoea grandifolia, and Eleusine indica*).

Lignin has been also reported as matrix for herbicides pelargonic acid and Citronella oil in nanoemulsion formulations to kill *Pennisetum purpureum* (Elephant grass) (Restu et al., 2023; Restu et al., 2024). Here, T*ridax procumbens* - coatbuttons. And after 28 days, seedlings presented lower values in fresh weight, and chorophyll levels (Figure 8A-D), apparently developing malfunction in germination/growth in the presence of spheres lignin nanoparticles. Of course, these results may be better evaluated in the future, approaching new concentration variables, time and post-emergence treatments. Although, it was important to highlight apparent changes caused by lignin nanoparticles and the value of future experiments using lignocellulose materials as main nanoparticles matrices.

4. Conclusion

Spherical lignin shapes were fabricated and carried herbicide atrazine by two possible routes: encapsulation and adsorption. Release and DFT experiments taken place and strong interactions between lignin and atrazine were notified. By hydroponic experiments, butterhead lettuces showed physiological changes with a sublethal dose of atrazine (30 µg/L). Lettuces treated with SLNPs and SLNPs_ATZ presented no significant changes in root length and shoot area when compared to control while lettuces treated with ATZ did. Biochemical tests such as enzymatic and relative gene expressions were assessed and showed up and down-regulated behaviors. Finally, weed seeds/seedlings had possible germination/development interferences caused by nanoherbicides - indicating lignin as a sustainable molecule capable of forming nanospheres that can load atrazine and effectively be used in future experiments with non-target/target plants with prospection of showing results in gene expression and control of weeds. Also, lignin can be arranged in multiple methods (encapsulation, modification, or in a mixture with other molecules) to enhance proprieties and give sustainability to new nanocarriers in agriculture. For future work, different concentrations in different nuances of lignin-nanoparticles must be explored to complement.

Author contributions

P.H.C.L.; M.C.S.B.; R.S.M and G.M.F: research, writing, review, and editing the manuscript. E.R.B, M.M.L.H.F and L.A.F.C research and review. R.G.: conceptualization, writing, review, editing, and supervision the manuscript. All authors have equally contributed to revising and reading the manuscript and have approved the submitted version.

Declaration of Competing Interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank National Council for Scientific and Technological Development, CNPq (grants # 310846/2022-6), São Paulo Research Foundation, FAPESP (grants #2021/03402-9, #2022/03219-2, #2023/00541-3, #2023/17369-9), and *The Coordination of Improvement of Higher Education Personnel* (CAPES - Finance Code 001). We also want to acknowledge the Emergency Support for Multi-User Equipment from São Paulo State University's Vice-Presidency of Research (PROPe), supporting the technical assistance from Maria Clara Shiroma Buri.

Author contributions

P.H.C.L., M.C.S.B., R.S.M and G.M.F: research, writing, review, and editing the manuscript. E.R.B, M.M.L.H.F and L.A.F.C research and review. R.G.: conceptualization, writing, review, editing, and supervision the manuscript. All authors have equally contributed to revising and reading the manuscript and have approved the submitted version.

Declaration of Competing Interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank National Council for Scientific and Technological Development, CNPq (grants # 310846/2022-6), São Paulo Research Foundation, FAPESP (grants #2021/03402-9, #2022/03219-2, #2023/00541-3, #2023/17369-9), and *The Coordination of Improvement of Higher Education Personnel* (CAPES - Finance Code 001). We also want to acknowledge the Emergency Support for Multi-User Equipment from São Paulo State University's Vice-Presidency of Research (PROPe), supporting the technical assistance from Maria Clara Shiroma Buri.

SUPPLEMENTARY MATERIAL

Text S1:

For SEM, 500 µL of solutions were dialyzed for 3 days and a drop was put in a grinding plate and dried in the desiccator. After analysis, the relative percentage frequency distribution was measured using Image J software with 600 nanoparticles in four images per formulation. For TGA and DSC, the material (SLNPs/SLNPs_ATZ) were put in petri dishes and oven-dried at 50ºC per 24 hours and stocked in a desiccator. Later, 5 to 7 mg of the oven dried material were put in clean TGA/ DSC aluminum hermetic pan. For FTIR, 1 mg of dried formulations were put together with 139 mg of potassium bromide (KBr) as an inert medium and pressed in around 2.7 tons.

Table S1 - Red flex fertilizer for hydroponics

Compounds	% for treatment
N	8
P_2O_5	8

K_2O	30
S	3
Mg	1
Fe	0.14
B	0.04
Mn	0.04
Cu	0.03
Zn	0.019
Mo	0.009
Ni	0.006
Co	0.002

Table S2 - Blue flex fertilizer for hydroponics

Compounds	% for treatment
N	10
Ca	15
Mg	2

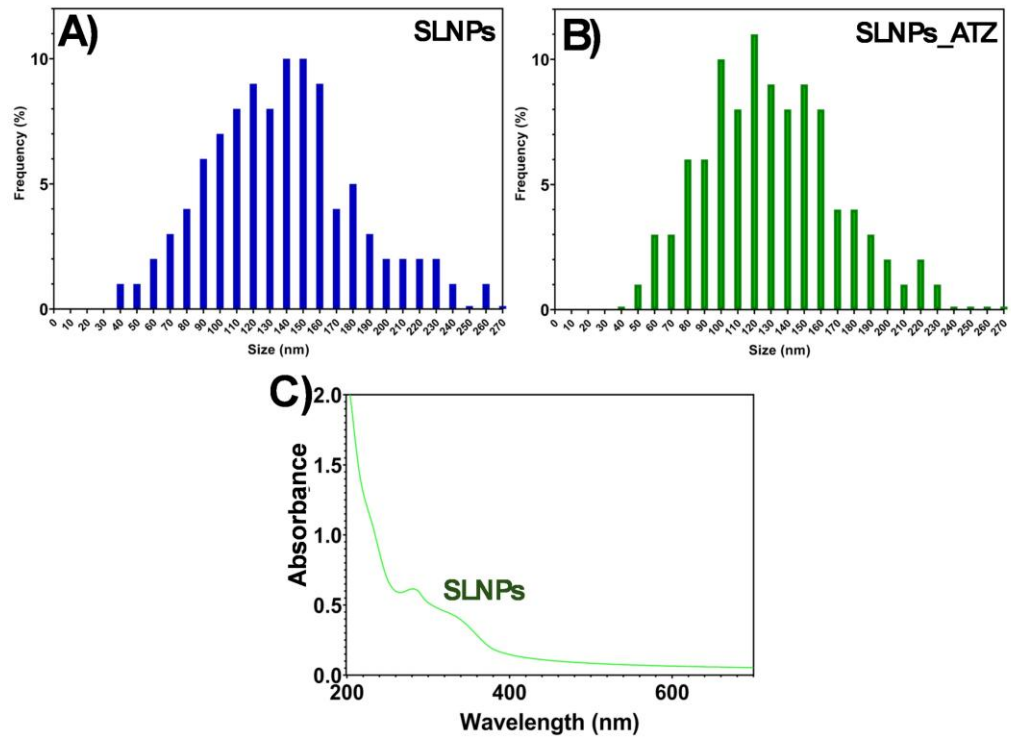


Figure S1 – (a) distribution frequency of SLNPs_ATZ and (b) ATZ, (c) and UV spectrum of spherical lignin nanoparticles.

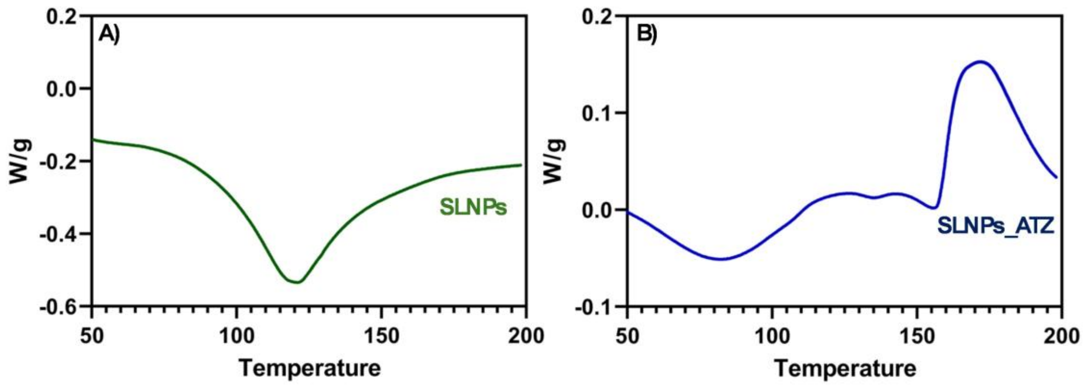


Figure S2- DSC analysis of SLNPs and SLNPs.

Table S3 – Regression values for various Kinect Moldes.

Kinect Model	R^2 (coefficient of determination)
Zero Order	0.7443
First Order	0.8002
Higuchi	0.9085
Korsmeyer-Peppas	0.9884
Hixon Crowell	0.7443

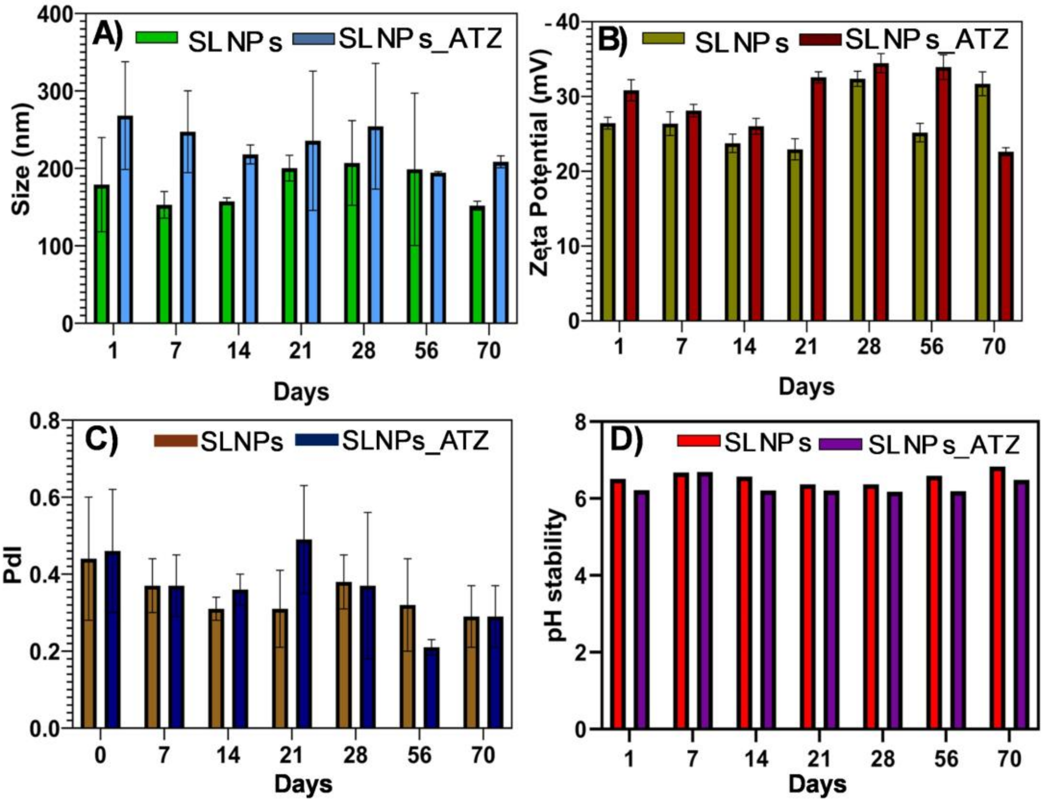


Figure S3- (a) size, (b) zeta potential and (c) PdI of SLNPs and SLNPs, and (d) pH stability of the nanoparticles over 70 days.

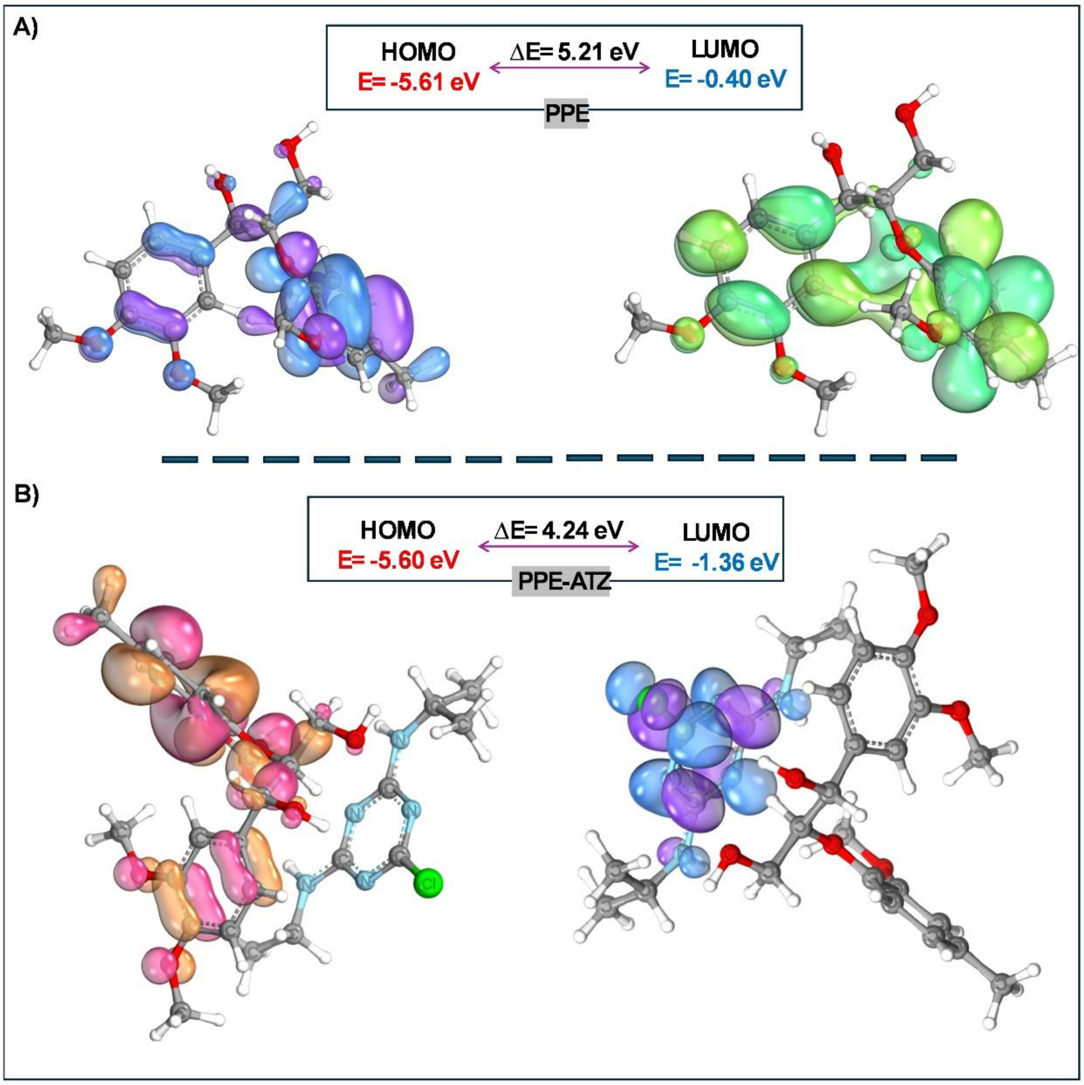


Figure S4 – DFT of ATZ and PPE.

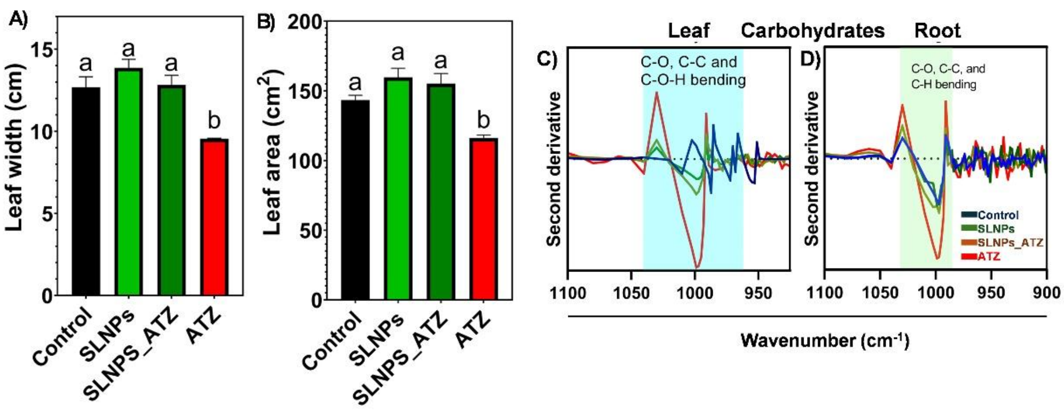


Figure S5 – (a) leaf width and (b) leaf area, (c) leaf and (d) root FTIR second derivative.

Table S4. Chlorophyll profile over time

Total Chlorophyll	**Control**		**SLNPS**		**SLNPs_ATZ**		**ATZ**	
	FCI	SD	FCI	SD	FCI	SD	FCI	SD
Day 0	18.7125	0.7875	19.0375	0.4875	19.525	0.61875	18.725	0.85
Day 14	18.35	0.5875	18.775	0.74375	17.35	0.7125	17.22857	0.795918
Day 28	20.3	0.55	20.325	0.275	18.05	0.85	17.075	0.2875
Chlorophyll a	**Control**		**SLNPS**		**SLNPs_ATZ**		**ATZ**	
	FCI	SD	FCI	SD	FCI	SD	FCI	SD
Day 0	15.48125	1.116406	15.53125	1.064844	15.925	1.196875	15.73125	1.201563
Day 14	15.45	1.125	15.7625	1.270313	14.8125	1.339063	14.1375	1.375
Day 28	16.13333	0.111111	16.63333	0.155556	14.43333	0.377778	14.06667	0.311111
Chlorophyll b	**Control**		**SLNPS**		**SLNPs_ATZ**		**ATZ**	
	FCI	SD	FCI	SD	FCI	SD	FCI	SD
Day 0	3.20625	0.307813	3.3375	0.35	3.36875	0.31015	3.20625	0.432031
Day 14	3.08125	0.328906	3.11875	0.283594	2.925	0.24687	2.8125	0.276563
Day 28	3.525	0.075	3.4	0.15	3.075	0.1875	2.775	0.125

CHAPTER III - **FINAL CONCLUSIONS, SCIENTIFIC-SOCIAL IMPACT AND RELEVANCE**

Final conclusions

Many controlled release systems for agrochemicals have been developed in recent years (Pontes et al., 2021; López-Cabeza et al., 2022). However, the design of smart nanocarriers that can be manufactured with renewable and low-cost materials is still a challenge for agricultural applications. Lignocellulosic materials (cellulose, lignin, and hemicellulose) may be ideal candidates for manufacturing green nanocarriers, given their low cost, abundance, and sustainability (Lima et al., 2021; Rodrigues et al., 2024). Lignin is a complex, three-dimensional aromatic molecule that is linked to cellulose and hemicellulose within the microstructure of the plant cell wall and is available on a large scale in agricultural waste (Lizundia et al., 2021). In recent years, lignin-based nanoparticles have been used in agriculture against fungi and insects due to their eco-friendly properties, low cost, and enhanced encapsulation properties (Ashok et al., 2018; Gigli et al., 2022).

In this thesis, the development of a new delivery system for the herbicide Atrazine (ATZ) was notified. Atrazine is a banned compound in European Union (but not in countries such as the United States and Brazil) since 2003 because its contamination and bioaccumulation affect soil and water, causing negative effects in non-target organisms and human (Wu et al., 2021; Pereira et al., 2024; Queiroz et al., 2024). For more efficient delivery systems, atrazine (ATZ) has a hydrophobic nature that can be correlated with nanoparticles with hydrophobic cores (Chandra et al., 2021; Song et al., 2024; Bryant et al., 2024). In this sense, lignin nanospheres (SLNPs) (Trevisan et al., 2020; Santo Pereira et al., 2022). – may strongly interact with atrazine. Understanding how nanoherbicides can affect non-target crops (e.g., lettuce) and more sensitive targets (weeds) is essential for risk assessment in agricultural products (Chen et al., 2024).

SLNPs have cavities in the center attributed to the π-π interaction due to benzene rings, on the other hand, the hydroxyl groups in the surface are hydrogen bonded with water (Trevisan et al., 2020; Santo Pereira et al., 2022). They have already been used to carry molecules such as resveratrol (Dai et al., 2017), but there are no reports of the use of these nanospheres in the carrying of atrazine (SLNPs_ATZ). The hypothesis of interaction between lignin and atrazine is unique with a two-way path: through adsorption and englobing (Wang et al., 2019). The nanospheres had nanometric sizes with a negative surface charge, and pH values around 6.22 and 6.51, respectively on the first day. On the seventieth, the results were 6.83 and 6.48. The encapsulation efficiency was 74.2%, suggesting an interaction between the lignin nanospheres and atrazine.

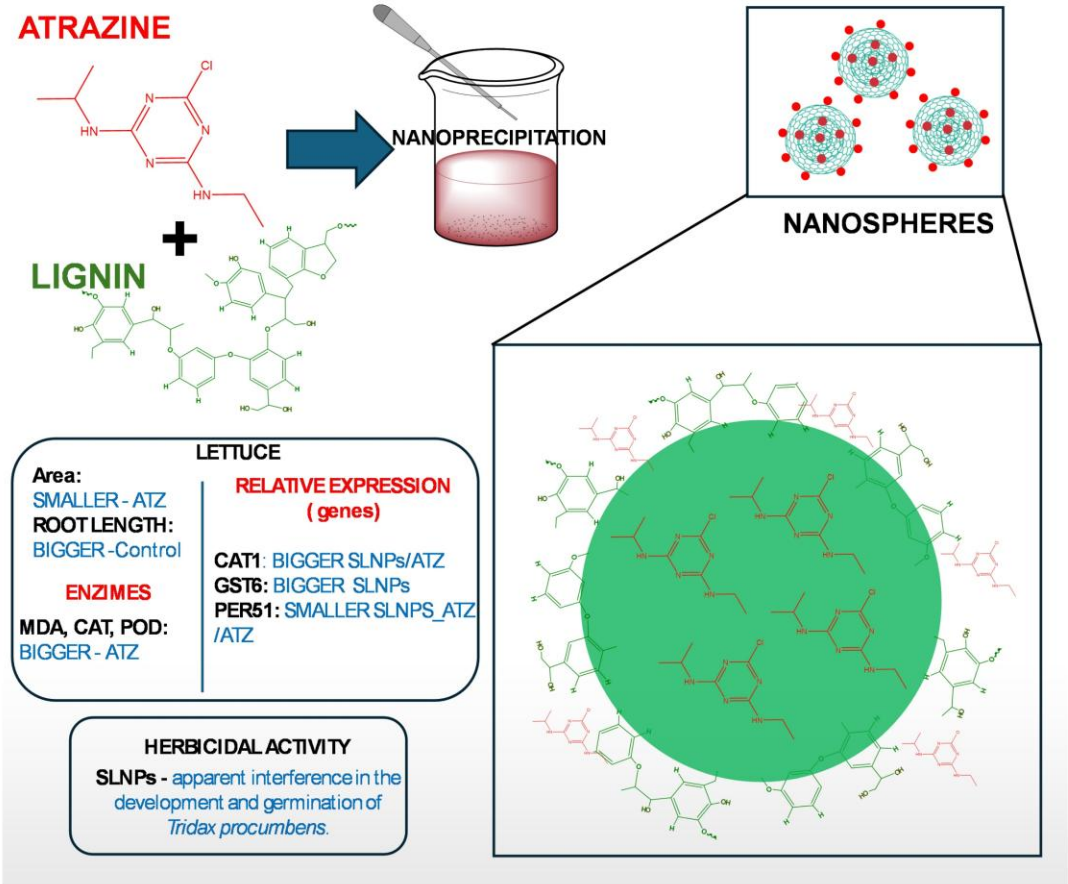


Figure 1 - Schematic representation of the lignin nanospheres containing atrazine and their interactions, and a summary of the results found in plants.

In the experiment with non-target plants (lettuce), there was a significant difference comparing root size and leaf/root area between atrazine (30 μg/L) and control, however, SLNPs and SLNPs_ATZ did not show significant changes when compared to the control. ROS species were also studied in lettuce after 28 days. Malondialdehyde (MDA) of lettuce exposed to atrazine did not reveal significant changes in the shoot, however, MDA of the roots showed significant differences comparing ATZ with all other treatments. On the other hand, Catalase (CAT) activity in the shoot of lettuce showed significant changes comparing ATZ with controls, while lettuce treated with SLNPs and SLNPs_ATZ had intermediate behavior. For guaiacol peroxidase, lettuce showed a significantly decreasing concentration in the root part (ATZ > SLNPs_ATZ > SLNPs > Control). By gene expression, the CAT1 genes were more highly expressed in lettuces treated with SLNPs and ATZ, and the GST6 genes were more highly expressed in lettuces treated with SLNPs. However, lettuces treated with SLNPs showed more physiological similarities to the control, indicating a possible tolerance of lettuces to nanoparticles. In addition, the PER51 genes were negatively expressed in plants treated with SLNPs_ATZ and ATZ, and there were no significant changes with SLNPs when compared to the control. This may have occurred because atrazine may have mutated or denatured the gene (Ramel et al., 2009). Finally, weed seeds/seedlings had possible interferences in germination/development when treated with SLNPs.

In conclusion, humanity has been trying to increase agricultural productivity with less damage to the environment. In recent years, nanotechnology has been complementing the release of active ingredients, and lignin – which comes from agricultural waste – has been indicated as a sustainable molecule in the construction of nanocarriers of agricultural bio-actives. In this context, lignin carrier nanospheres with atrazine (herbicide) were manufactured and used effectively in experiments with non-target/target plants, showing possible results in gene expression and weed control. This work will serve as a model for future studies that seek to improve sustainability and reduce the quantity of herbicides used in the field. Furthermore, in the future, lignin can be explored with other methods (encapsulation, modification, or mixture with other molecules) in the nanocarrier of herbicides (Abbas et al., 2025), aiming to increase properties and giving sustainability to new nanocarriers in agriculture.

SCIENTIFIC-SOCIAL IMPACT AND RELEVANCE

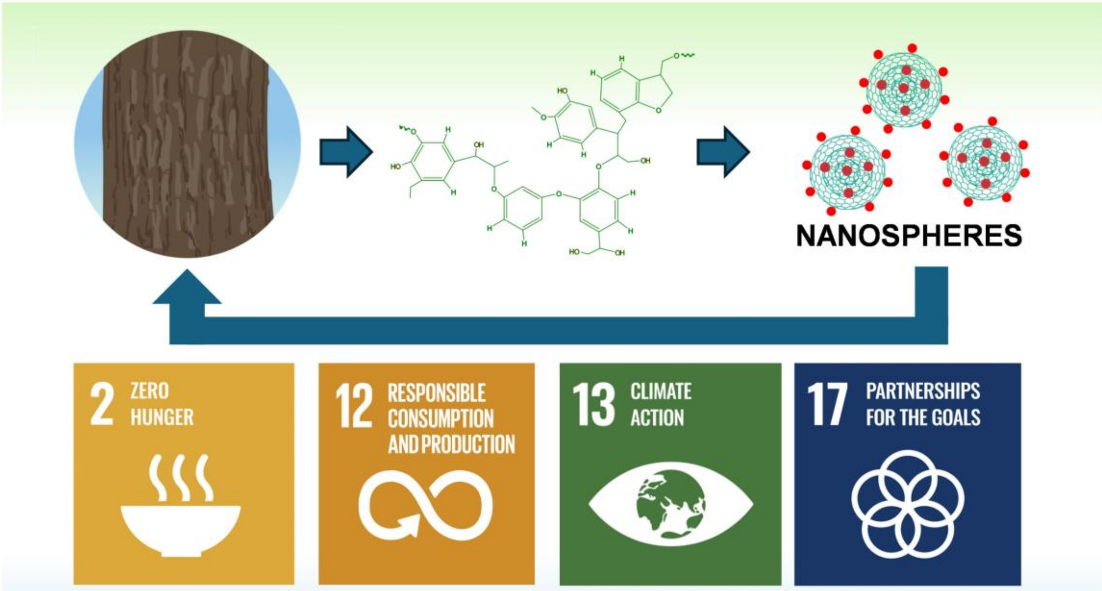


Figure 2 – Sustainable cycle scheme of lignin nanospheres and SDGs targets: number 2, 12, 13 and 17.

This technical-scientific work can be attributed to the production of new knowledge in the development of nanoparticle systems for the release of bio-actives for modern agriculture. The scientific, social, and environmental impacts in our study corroborate the Sustainable Development Goals (SDGs) established by the United Nations (UN) (Figure 2) (Cf, 2015). SDG target number 2 is reflected in our work as it advocates “End hunger, achieve food security and improve nutrition and promote sustainable agriculture” and we developed a system that promotes sustainability using a molecule derived from agricultural input and returning to the production cycle. Our system is also aligned with target number 12 “Ensure sustainable consumption and production patterns” because it is sustainable - from lignocellulosic materials.

Another highlighted goal is number 13 - take urgent action to combat climate change and its impacts – where the excessive use of herbicides causes irreparable damage to fauna, flora, and human health. This work sought to reduce the quantity of herbicides in a sustainable manner and tried to mitigate the impact of them on soil and water. In addition, goal 17 is also reflected in our research, since according to topic 17.14 "Increase policy coherence for sustainable development", this work fits this criterion. So, this work can serve as an example for future researchers, stimulating the exploration of new controlled-release nanocarriers in different agricultural contexts, contributing to the reduction of environmental impacts while increasing agricultural production.

SCIENTIFIC PRODUCTION RELATED TO THE THESIS

Published articles:

de Lima, P. H. C., Tavares, A. A., de Lima Silva, S. M., de Moura, M. R., Aouada, F. A., and Grillo, R. (2022). Recent advances on nanohybrid systems constituting clay–chitosan with organic molecules–a review. *Applied Clay Science*, *226*, 106548.

Forini, M. M., Pontes, M. S., Antunes, D. R., **de Lima, P. H**., Santos, J. S., Santiago, E. F., and Grillo, R. (2022). Nano-enabled weed management in agriculture: From strategic design to enhanced herbicidal activity. *Plant Nano Biology*, *1*, 100008.

de Lima, P. H. C., Ribeiro-Viana, R. M., Plath, A. M. S., and Grillo, R. (2024). Lignocellulosic-biomolecules conjugate systems: green-engineered complexes modified by covalent linkers. *Journal of Materials Chemistry B*.

Antunes, D. R., Forini, M. M., Coqueiro, Y. A., Pontes, M. S., **Lima, P. H.,** Cavalcante, L. A., and Grillo, R. (2024). Effect of hyaluronic acid-stabilized silver nanoparticles on lettuce (Lactuca sativa L.) seed germination. *Chemosphere*, *364*, 143080.

REFERENCES

Abbas, A., Lai, D. Y., Peng, P., and She, D. (2025). Lignin-Based Functional Materials in Agricultural Application: A Review. *Journal of Agricultural and Food Chemistry*, *73*(10), 5685-5710.

Abbati de Assis, C., Greca, L. G., Ago, M., Balakshin, M. Y., Jameel, H., Gonzalez, R., and Rojas, O. J. (2018). Techno-economic assessment, scalability, and applications of aerosol lignin micro-and nanoparticles. *ACS sustainable chemistry and engineering*, 6(9), 11853-11868.

Acharya, A., and Pal, P. K. (2020). Agriculture nanotechnology: Translating research outcome to field applications by influencing environmental sustainability. *NanoImpact*, 19, 100232.

Agathokleous, E., Feng, Z., Iavicoli, I., and Calabrese, E. J. (2020). Nano-pesticides: A great challenge for biodiversity? The need for a broader perspective. *Nano Today*, 30, 100808.

Agustin, M. B., Penttilä, P.A., Lahtinen, M., and Mikkonen, K. S. (2019). Rapid and direct preparation of lignin nanoparticles from alkaline pulping liquor by mild ultrasonication. *ACS Sustainable Chemistry and Engineering*, 7(24), 19925–19934.

Ahmad, U. M.; Ji, N.; Li, H.; Wu, Q.; Song, C.; Liu, Q.; and Lu, X. Can lignin be transformed into agrochemicals? Recent advances in the agricultural applications of lignin. (2021). Industrial Crops and Products 170, 113646.

Aijaz, N., Lan, H., Raza, T., Yaqub, M., Iqbal, R., and Pathan, M. S. (2025). Artificial Intelligence in Agriculture: Advancing Crop Productivity and Sustainability. Journal of Agriculture and Food Research, 101762.

Akpinar, I.; Drout, R. J.; Islamoglu, T.; Kato, S.; Lyu, J.; and Farha, O. K. Exploiting π–π interactions to design an efficient sorbent for atrazine removal from water. (2019). ACS Appl. Mater. Interfaces 11(6), 6097-6103.

Alscher, R. G.; Erturk, N.; Heath, L. S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot., 53(372), 1331-1341.

An, J.; Hu, P.; Li, F.; Wu, H.; Shen, Y.; White, J. C.; Giraldo, J. P. (2020). Emerging investigator series: molecular mechanisms of plant salinity stress tolerance improvement by seed priming with cerium oxide nanoparticles. Environ. Sci. Nano, 7(8), 2214-2228.

Antunes, D. R.; Forini, M. M.; Coqueiro, Y. A.; Pontes, M. S.; Lima, P. H.; Cavalcante, L. A.; and Grillo, R. Effect of hyaluronic acid-stabilized silver nanoparticles on lettuce (Lactuca sativa L.) seed germination. (2024). Chemosphere 364, 143080.

Anushikha; Gaikwad, K. K. Lignin as a UV blocking, antioxidant, and antimicrobial agent for food packaging applications. (2024). Biomass Conversion and Biorefinery 14(15), 16755-16767.

Ashok, R. P. B., Oinas, P., Lintinen, K., Sarwar, G., Kostiainen, M. A., and Österberg, M. (2018). Techno-economic assessment for the large-scale production of colloidal lignin particles. *Green Chemistry*, 20(21), 4911-4919.

Azevedo, R. A.; Alas, R. M.; Smith, R. J.; and Lea, P. J. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. (1998). Physiologia Plantarum 104(2), 280-292.

Bahloul, A., Kassab, Z., El Bouchti, M., Hannache, H., Oumam, M., and El Achaby, M. (2021). Micro-and nano-structures of cellulose from eggplant plant (Solanum melongena L) agricultural residue. *Carbohydrate Polymers*, 253, 117311.

Baker, S., Volova, T., Prudnikova, S. V., Satish, S., and Prasad, N. (2017). Nanoagroparticles emerging trends and future prospect in modern agriculture system. *Environmental toxicology and pharmacology*, 53, 10-17.

Balakshin, M., and Capanema, E. (2015). On the quantification of lignin hydroxyl groups with 31P and 13C NMR spectroscopy. *Journal of Wood Chemistry and Technology*, 35(3), 220-237.

Bauli, C. R., Lima, G. F., de Souza, A. G., Ferreira, R. R., and Rosa, D. S. (2021). Eco-friendly carboxymethyl cellulose hydrogels filled with nanocellulose or nanoclays for agriculture applications as soil conditioning and nutrient carrier and their impact on cucumber growing. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 623, 126771.

Beckers, S. J., Fischer, J., and Wurm, F. R. (2021). Synthetic lignin-like and degradable nanocarriers. *Polymer Chemistry*, 12(32), 4661-4667.

Beckers, S. J., Wetherbee, L., Fischer, J., and Wurm, F. R. (2020a). Fungicide-loaded and biodegradable xylan-based nanocarriers. *Biopolymers*, 111(12), e23413.

Beckers, S., Peil, S., and Wurm, F. R. (2020b). Pesticide-Loaded Nanocarriers from Lignin Sulfonates—A Promising Tool for Sustainable Plant Protection. *ACS sustainable chemistry and engineering*, 8(50), 18468-18475.

Behera, S.; Mohapatra, S.; Behera, B. C.; and Thatoi, H. Recent updates on green synthesis of lignin nanoparticle and its potential applications in modern biotechnology. (2024). Critical Reviews in Biotechnology 44(5), 774-794.

Behtash, F.; Amini, T.; Mousavi, S. B.; Seyed Hajizadeh, H.; and Kaya, O. Efficiency of zinc in alleviating cadmium toxicity in hydroponically grown lettuce (Lactuca sativa L. cv. Ferdos). (2024). BMC Plant Biology 24(1), 648.

Belakhdar, A.; Ferkous, H.; Djellali, S.; Sahraoui, R.; Lahbib, H.; Amor, Y. B.; and Benguerba, Y. Computational and experimental studies on the efficiency of Rosmarinus officinalis polyphenols as green corrosion inhibitors for XC48 steel in acidic medium. (2020). Colloids and Surfaces A: Physicochemical and Engineering Aspects 606, 125458.

Ben Haj Fraj, S.; Chaabene, M.; Agren, S.; El Haskouri, J.; Lahcini, M.; Ben Chaâbane, R.; Baouab, M. H. V. High incorporation of magnetite nanoparticles inside tetraaza macrocyclic Schiff base cavity: spectroscopic characterization and modeling by DFT calculation. (2022). Journal of the Iranian Chemical Society 19(12), 4803-4822.

Bhattacharyya, C., Roy, R., Tribedi, P., Ghosh, A., and Ghosh, A. (2020). Biofertilizers as substitute to commercial agrochemicals. In Agrochemicals Detection, Treatment and Remediation, 263-290. *Butterworth-Heinemann*.

Bigott, Y.; Chowdhury, S. P.; Pérez, S.; Montemurro, N.; Manasfi, R.; and Schröder, P. Effect of the pharmaceuticals diclofenac and lamotrigine on stress responses and stress gene expression in lettuce (Lactuca sativa) at environmentally relevant concentrations. (2021). Journal of Hazardous Materials 403, 123881.

Brovini, E. M., Quadra, G. R., Paranaíba, J. R., Carvalho, L., de Oliveira Pereira, R., and de Aquino, S. F. (2023). Occurrence and environmental risk assessment of 22 pesticides in Brazilian freshwaters. *Aquatic Toxicology*, *260*, 106566.

Bryant, M. T.; Ren, J.; Sharma, V. K.; and Ma, X. Mutual Effects and Uptake of Organic Contaminants and Nanoplastics by Lettuce in Co-Exposure. (2024). ACS Agricultural Science and Technology 4(4), 463-470.

Carvalho, L. B.; Godoy, I. S.; Preisler, A. C.; de Freitas Proença, P. L.; Saraiva-Santos, T.; Verri, W. A.; Fraceto, L. F. Pre-emergence herbicidal efficiency and uptake of atrazine-loaded zein nanoparticles: a sustainable alternative to weed control. (2023). Environmental Science: Nano 10(6), 1629-1643.

Cf, O. D. D. S. (2015). Transforming our world: the 2030 Agenda for Sustainable Development. *United Nations, New York*.

Chamundeeswari, M., Jeslin, J., and Verma, M. L. (2019). Nanocarriers for drug delivery applications. *Environmental Chemistry Letters*, 17(2), 849-865.

Chance, B.; and Maehly, A. C. Assay of catalases and peroxidases. (1955).

Chandra, P. N.; and Usha, K. Removal of atrazine herbicide from water by polyelectrolyte multilayer membranes. (2021). Materials Today: Proceedings 41, 622-627.

Chen, L., Zhou, H., Hao, L., Li, Z., Xu, H., Chen, H., and Zhou, X. (2020b). Dialdehyde carboxymethyl cellulose-zein conjugate as water-based nanocarrier for improving the efficacy of pesticides. *Industrial Crops and Products,* 150, 112358.

Chen, S., Ye, Y., Liao, F., Wu, S., and Zhang, K. (2024). Insight into the uptake, translocation, metabolism, dissipation and risk assessment of tolfenpyrad in romaine and edible amaranth grown in hydroponic conditions. Food Chemistry, 437, 137896.

Chen, Z., Ragauskas, A., and Wan, C. (2020a). Lignin extraction and upgrading using deep eutectic solvents. *Industrial Crops and Products*, 147, 112241.

Cheng, B.; Ding, Z.; Yue, L.; Chen, F.; Cao, X.; Li, J.; Wang, Z. (2023). Carbon dots enhanced cold tolerance of lettuce (Lactuca sativa L.): scavenging reactive oxygen species, modulating hormones and up-regulating gene expression. Environ. Sci. Nano, 10(10), 2849-2860.

Cheng, J.; Li, M.; Zhu, R. X.; Li, J. Y.; Yin, J.; and Xiao, W. A timing self-cleaning nanoherbicide: Design of triple-structure nanovectors for weed control and pesticide residues treatment. (2024). Advanced Agrochem 3(4), 298-307.

Cheng, S.; Wang, J.; Yang, J.; Wang, X.; Tang, J. An effective strategy to design high-performance wide bandgap polymer donors for organic solar cells by increasing the backbone curvature. (2023a). Dyes and Pigments 219, 111626.

Chikoye, D.; Udensi, U. E.; Lum, A. F. (2005). Evaluation of a new formulation of atrazine and metolachlor mixture for weed control in maize in Nigeria. Crop Protection, 24(11), 1016-1020.

Chowdhury, S. P.; Babin, D.; Sandmann, M.; Jacquiod, S.; Sommermann, L.; Sørensen, S. J.; and Grosch, R. (2019). Effect of long-term organic and mineral fertilization strategies on rhizosphere microbiota assemblage and performance of lettuce. Environmental Microbiology 21(7), 2426-2439.

Chowdhury, S. P.; Uhl, J.; Grosch, R.; Alquéres, S.; Pittroff, S.; Dietel, K.; and Hartmann, A. (2015). Cyclic lipopeptides of Bacillus amyloliquefaciens subsp. plantarum colonizing the lettuce rhizosphere enhance plant defense responses toward the bottom rot pathogen Rhizoctonia solani. Molecular Plant-Microbe Interactions 28(9), 984-995.

Chowdhury, M.; Samarakoon, U. C.; and Altland, J. E. Evaluation of hydroponic systems for organic lettuce production in controlled environment. (2024). Frontiers in Plant Science 15, 1401089.

Cordon, G.; Valino, I. L.; Prieto, A.; Costa, C.; Marchi, M. C.; and Diz, V. Effects of the nanoherbicide made up of atrazine-chitosan on the primary events of photosynthesis. (2022). Journal of Photochemistry and Photobiology 12, 100144.

Credou, J., and Berthelot, T. (2014). Cellulose: from biocompatible to bioactive material. *Journal of Materials Chemistry B*, 2(30), 4767-4788.

da Silva Santos, J.; da Silva Pontes, M.; Grillo, R.; Fiorucci, A. R.; de Arruda, G. J.; Santiago, E. F.; and [add if missing last author]. Physiological mechanisms and phytoremediation potential of the macrophyte Salvinia biloba towards a commercial formulation and an analytical standard of glyphosate. (2020). Chemosphere 259, 127417.

Dai, L.; Liu, R.; Hu, L. Q.; Zou, Z. F.; and Si, C. L. Lignin nanoparticle as a novel green carrier for the efficient delivery of resveratrol. (2017). ACS Sustainable Chemistry and Engineering 5(9), 8241-8249.

Darbyshire, M.; Coutts, S.; Bosilj, P.; Sklar, E.; and Parsons, S. (2024). Review of weed recognition: A global agriculture perspective. *Comput. Electron. Agric*, *227*, 109499.

Das, D., Prakash, P., Rout, P. K., and Bhaladhare, S. (2021). Synthesis and Characterization of Superabsorbent Cellulose-Based Hydrogel for Agriculture Application. *Starch-Stärke*, 73(1-2), 1900284.

De Oliveira, F. K.; Santos, L. O.; Buffon, J. G. (2021). Mechanism of action, sources, and application of peroxidases. Food Res. Int., 143, 110266.

de Souza, V. V.; da Silva Souza, T.; de Campos, J. M. S.; de Oliveira, L. A.; Ribeiro, Y. M.; de Melo Hoyos, D. C.; and Lacerda, S. M. D. S. N. Ecogenotoxicity of environmentally relevant atrazine concentrations: a threat to aquatic bioindicators. (2023). Pestic. Biochem. Physiol. 189, 105297.

Debaeke, P.; Perronne, R.; Colbach, N.; Moreau, D.; Barre, P.; Lecouviour, F.; and Durand-Tardif, M. (2024). Non-chemical weed management: Which crop functions and traits to improve through breeding? *Crop Prot.*, 106631.

Del Buono, D.; Luzi, F.; Puglia, D. (2021). Lignin nanoparticles: A promising tool to improve maize physiological, biochemical, and chemical traits. Nanomaterials, 11(4), 846.

Dghoughi, A.; Raji, M.; Chakchak, H.; Bensalah, M. O.; Bouhfid, R.; and Qaiss, A. E. K. Synthesis of triangular lignin photonic crystal nanoparticles: Investigating solvent effects and dialysis optimization. (2025). Int. J. Biol. Macromol. 291, 139110.

Di, X.; Fu, Y.; Xu, Y.; Zheng, S.; Huang, Q.; Sun, Y. (2023). Assessment of CuO NPs on soil microbial community structure based on phospholipid fatty acid techniques and phytotoxicity of bok choy seedlings. Plant Physiol. Biochem., 197, 107657.

do Nascimento, D. M., Nunes, Y. L., de Almeida, J. S., Leitão, R. C., Feitosa, J., Dufresne, A., and Rosa, M. D. F. (2021). Development of an integrated process to produce CNFs and lignin and its potential applications for agrochemical delivery. *Cellulose*, 1-14.

Dong, J.; Liu, X.; Chen, Y.; Yang, W.; and Du, X. User-safe and efficient chitosan-gated porous carbon nanopesticides and nanoherbicides. (2021a). J. Colloid Interface Sci. 594, 20–34.

Dong, J., Chen, W., Feng, J., Liu, X., Xu, Y., Wang, C., et al. (2021b). Facile, Smart, and Degradable Metal–Organic Framework Nanopesticides Gated with FeIII-Tannic Acid Networks in Response to Seven Biological and Environmental Stimuli. *ACS Appl. Mater. Inter.* 13 (16), 19507–19520.

Doyle, J. J.; and Doyle, J. L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. (1987). Phytochem. Bull.

Eghbalinejad, M.; Hofman, J.; Kotouček, J.; Grillo, R.; Hochmanová Bílková, Z.; Reiff, N.; and Höss, S. Nano-enabled pesticides: a comprehensive toxicity assessment of tebuconazole nanoformulations with nematodes at single species and community level. (2024). Environ. Sci. Eur. 36, 51.

Ela, R. C. A., Tajiri, M., Newberry, N. K., Heiden, P. A., and Ong, R. G. (2020). Double-shell lignin nanocapsules are a stable vehicle for fungicide encapsulation and release. *ACS Sustainable Chemistry and Engineering*, 8(46), 17299-17306.

Falsini, S., Clemente, I., Papini, A., Tani, C., Schiff,S., Salvatici, M. C., Petruccelli, R., Benelli, C., Giordano, G., Gonnelli, C. and Ristori, S. (2019) When Sustainable Nanochemistry Meets Agriculture: Lignin Nanocapsules for Bioactive Compound Delivery to Plantlets. *ACS Sustainable Chemistry and Engineering*, 7 (24), 19935-19942.

Falsini, S., Tani, C., Schiff, S., Gonnelli, C., Clemente, I., Ristori, S., and Papini, A. (2020). A new method for the direct tracking of in vivo lignin nanocapsules in Eragrostis tef (Poaceae) tissues. *European journal of histochemistry: EJH*, 64(2).

Fischer, J., Beckers, S.J., Yiamsawas, D., Thines, E., Landfester, K. and Wurm, F.R. (2019). Targeted drug delivery in plants: enzyme-responsive lignin nanocarriers for the curative treatment of the worldwide grapevine trunk disease Esca. *Advanced Science*, 6, 1802315.

Forini, M. M.; Antunes, D. R.; Cavalcante, L. A.; Pontes, M. S.; Biscalchim, E. R.; Sanches, A. O.; and Grillo, R. Fabrication and characterization of a novel herbicide delivery system with magnetic collectability and its phytotoxic effect on photosystem II of aquatic macrophyte. (2020). J. Agric. Food Chem. 68(40), 11105–11113.

Forini, M. M.; Pontes, M. S.; Antunes, D. R.; de Lima, P. H.; Santos, J. S.; Santiago, E. F.; and Grillo, R. Nano-enabled weed management in agriculture: From strategic design to enhanced herbicidal activity. (2022). Plant Nano Biol. 1, 100008.

Fotovvat, M.; Najafi, F.; Khavari-Nejad, R. A.; Talei, D.; and Rejali, F. Investigating the simultaneous effect of chitosan and arbuscular mycorrhizal fungi on growth, phenolic compounds, PAL enzyme activity and lipid peroxidation in Salvia nemorosa L. (2024). Plant Physiol. Biochem. 210, 108617.

Fraceto, L. F.; Grillo, R.; de Medeiros, G. A.; Scognamiglio, V.; Rea, G.; Bartolucci, C. (2016). Nanotechnology in agriculture: which innovation potential does it have? *Front. Environ. Sci.*, *4*, 186737.

Freitas, F. M.; Cerqueira, M. A.; Gonçalves, C.; Azinheiro, S.; Garrido-Maestu, A.; Vicente, A. A.; Michelin, M. Green synthesis of lignin nano- and micro-particles: Physicochemical characterization, bioactive properties and cytotoxicity assessment. (2020). Int. J. Biol. Macromol. 163, 1798–1809.

Gajendra, G.; Pulimi, M.; Natarajan, C.; and Mukherjee, A. Occurrence, Toxicodynamics, and Mechanistic Insights for Atrazine Degradation in the Environment. (2024). Water Air Soil Pollut. 235(10), 649.

Gao, Y., Liu, Y., Qin, X., Guo, Z., Li, D., Li, C., and He, S. (2021a). Dual stimuli-responsive fungicide carrier based on hollow mesoporous silica/hydroxypropyl cellulose hybrid nanoparticles. *Journal of Hazardous Materials*, 414, 125513.

Gao, X., Fan, S., Pang, J., Rahman, M. Z., Zhu, D., Guo, S., Ma, M., and Li, Z. (2021b). Preparation of nano-xylan and its influences on the anti-fungi performance of straw fiber/HDPE composite. *Industrial Crops and Products,* 171, 113954.

Gautam, T. R.; Vasmatkar, P.; Gundloori, R. V.; Kadoo, N. Ethyl cellulose-based controlled-release atrazine nanoformulation for effective and long-term weed management in agriculture. (2025). Industrial Crops and Products 229, 120992.

Gazzurelli, C., Migliori, A., Mazzeo, P. P., Carcelli, M., Pietarinen, S., Leonardi, G., and Pelagatti, P. (2020). Making agriculture more sustainable: an environmentally friendly approach to the synthesis of lignin@ Cu pesticides. *ACS Sustainable Chemistry and Engineering*, 8(39), 14886-14895.

Ge, M.; Li, Y.; Zhu, C.; Liang, G.; SM, J. A.; Hu, G.; Gui, Y. Preparation of organic-modified magadiite–magnetic nanocomposite particles as an effective nanohybrid drug carrier material for cancer treatment and its properties of sustained release mechanism by Korsmeyer–Peppas kinetic model. (2021). J. Mater. Sci. 56(25), 14270–14286.

Ghazi, M. R., Nik Yusoff, N. R., Abdul Halim, N. S., Wahab, I. R. A., Ab Latif, N., Hasmoni, S. H., and Zakaria, Z. A. (2023). Health effects of herbicides and its current removal strategies. Bioengineered, 14(1), 2259526

Ghosh, A.; Kumar, A.; and Biswas, G (2024). Exponential population growth and global food security: challenges and alternatives. *In Bioremediation of Emerging Contaminants from Soils*, 1-20.

Gigli, M., Fellet, G., Pilotto, L., Sgarzi, M., Marchiol, L., and Crestini, C. (2022). Lignin-based nano-enabled agriculture: A mini-review. Frontiers in Plant Science, 13, 976410.

Gohar, F.; Iqbal, U. Z.; Khan, M. B.; Rehman, F.; Maryam, F. S. U.; Azmat, M.; Shahid, M. U. (2024). Impact of Nanoparticles on Plant Growth, Development and Physiological Processes: A Comprehensive Review.

Gong, Y.; Li, S.; Hu, Y.; Jin, G.; Zhou, H.; Lim, L. T.; Xiao, Q. Acidic and alkaline deep eutectic solvents pretreatment of Macadamia nutshells for production of cellulose nanofibrils and lignin nanoparticles. (2025). International Journal of Biological Macromolecules, 140251.

Grillo, R.; dos Santos, N. Z. P.; Maruyama, C. R.; Rosa, A. H.; de Lima, R.; Fraceto, L. F. Poly (ε-caprolactone) nanocapsules as carrier systems for herbicides: Physico-chemical characterization and genotoxicity evaluation. (2012). Journal of hazardous materials 231, 1-9.

Grillo, R.; Pereira, A. E.; Nishisaka, C. S.; De Lima, R.; Oehlke, K.; Greiner, R.; Fraceto, L. F. Chitosan/tripolyphosphate nanoparticles loaded with paraquat herbicide: an environmentally safer alternative for weed control. (2014). Journal of hazardous materials 278, 163-171.

Grillo, R., Clemente, Z., de Oliveira, J. L., Campos, E. V. R., Chalupe, V. C., Jonsson, C. M., and Fraceto, L. F. (2015). Chitosan nanoparticles loaded the herbicide paraquat: the influence of the aquatic humic substances on the colloidal stability and toxicity. *Journal of hazardous materials*, 286, 562-572.

Grillo, R.; Mattos, B. D.; Antunes, D. R.; Forini, M. M. L.; Monikh, F. A.; and Rojas, O. J. Foliage Adhesion and Interactions with Particulate Delivery Systems for Plant Nanobionics and Intelligent Agriculture. (2021a). Nano Today 37, 101078.

Grillo, R.; Fraceto, L. F.; Amorim, M. J. B.; Scott-Fordsmand, J. J.; Schoonjans, R.; and Chaudhry, Q. Ecotoxicological and Regulatory Aspects of Environmental Sustainability of Nanopesticides. (2021b). J. Hazard. Mater. 404, 124148.

Grillo, R.; Abhilash, P. C.; and Fraceto, L. F. Nanotechnology applied to bio-encapsulation of pesticides. (2016). *Journal of nanoscience and nanotechnology 16*(1), 1231-1234.

Guo, J., Tardy, B. L., Christofferson, A. J., Dai, Y., Richardson, J. J., Zhu, W., and Caruso, F. (2016). Modular assembly of superstructures from polyphenol-functionalized building blocks. *Nature nanotechnology*, *11*(12), 1105-1111.

Guo, S., He, F., Song, B., and Wu, J. (2021). Future direction of agrochemical development for plant disease in China. *Food and Energy Security*, e293.

Guo, Y.; Xu, L.; Shen, F.; Hu, J.; Huang, M.; He, J.; Tian, D. Insights into lignocellulosic waste fractionation for lignin nanospheres fabrication using acidic/alkaline deep eutectic solvents. (2022). Chemosphere 286, 131798.

Gupta, S. D.; Agarwal, A.; Pradhan, S. (2018). Phytostimulatory effect of silver nanoparticles (AgNPs) on rice seedling growth: An insight from antioxidative enzyme activities and gene expression patterns. Ecotoxicol. Environ. Saf., 161, 624–633.

Hao, L., Lin, G., Lian, J., Chen, L., Zhou, H., Chen, H., and Zhou, X. (2020). Carboxymethyl cellulose capsulated zein as pesticide nano-delivery system for improving adhesion and anti-UV properties. *Carbohydrate polymers*, 231, 115725.

Harper, A. R.; Dobson, R. C.; Morris, V. K.; Moggré, G. J. (2022). Fermentation of plant-based dairy alternatives by lactic acid bacteria. Microb. Biotechnol., 15(5), 1404–1421.

Hellal, A.; Abdelsalam, H.; Tawfik, W.; and Ibrahim, M. A. Removal of Atrazine from contaminated water by functionalized graphene quantum dots. (2024a). Opt. Quantum Electron. 56(3), 374.

Hellal, A.; Abdelsalam, H.; Tawfik, W.; Ibrahim, M. A. Assessment of doped graphene in the removal of atrazine from water. (2024b). Sci. Rep. 14(1), 21994.

Hennig, T. B.; Bandeira, F. O.; Puerari, R. C.; Fraceto, L. F.; Matias, W. G. A systematic review of the toxic effects of a nanopesticide on non-target organisms: Estimation of protective concentrations using a species sensitivity distribution (SSD) approach–The case of atrazine. (2023). Sci. Total Environ. 871, 162094.

Hoffmann, G.; Chermette, H.; Morell, C. Revisiting nucleophilicity: an index for chemical reactivity from a CDFT approach. (2024). J. Mol. Model. 30(7), 232.

Houston, R. W.; Elder, T. J.; Abdoulmoumine, N. H. Investigation into the pyrolysis bond dissociation enthalpies (BDEs) of a model lignin oligomer using density functional theory (DFT). (2022). Energy Fuels 36(3), 1565–1573.

Jiang, Y., Chen, Y., Tian, D., Shen, F., Wan, X., Xu, L., and Shen, F. (2020). Fabrication and characterization of lignin–xylan hybrid nanospheres as pesticide carriers with enzyme-mediated release property. *Soft Matter*, 16(39), 9083-9093.

Jiang, D.; Jiang, Z.; Liu, S.; Hu, Y.; Deng, S.; Wang, J.; Zhang, Y. Inhibition mechanism of atrazine on soybean growth insight from the plant nitrogen fixation and rhizobia diversity inhabiting in nodules and rhizosphere soil. (2024). Appl. Soil Ecol. 195, 105236.

Jiang, Z.; Shao, Q.; Chu, Y.; An, N.; Cao, B.; Ren, Z.; and Zhang, Y. Mitigation of atrazine-induced oxidative stress on soybean seedlings after co-inoculation with atrazine-degrading bacterium Arthrobacter sp. DNS10 and inorganic phosphorus-solubilizing bacterium Enterobacter sp. P1. (2023a). Environ. Sci. Pollut. Res. 30(11), 30048–30061.

Jiang, D.; Li, Y.; Wang, J.; Lv, X.; Jiang, Z.; Cao, B.; Zhang, Y. Exogenous application of Bradyrhizobium japonicum AC20 enhances soybean tolerance to atrazine via regulating rhizosphere soil microbial community and amino acid, carbohydrate metabolism related genes expression. (2023b). Plant Physiol. Biochem. 196, 472–483.

Jin, Y.; Yuan, X.; Lu, Y.; Teng, G.; Zhou, C.; Wang, Y.; and Zhang, J. A Self-Reportable Fluorescence Lighting-up Nanoherbicide with Minimized Off-Target Hazards. (2025). Adv. Agrochem.

Jogaiah, S., Paidi, M. K., Venugopal, K., Geetha, N., Mujtaba, M., Udikeri, S. S., and Govarthanan, M. (2021). Phytotoxicological effects of engineered nanoparticles: An emerging nanotoxicology. *Science of the Total Environment*, 149809.

Jomova, K.; Raptova, R.; Alomar, S. Y.; Alwasel, S. H.; Nepovimova, E.; Kuca, K.; Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Arch. Toxicol., 97(10), 2499–2574.

Kamble, S.; Agrawal, S.; Cherumukkil, S.; Sharma, V.; Jasra, R. V.; Munshi, P. Revisiting zeta potential, the key feature of interfacial phenomena, with applications and recent advancements. (2022). ChemistrySelect 7(1), e202103084.

Kaya, M., and Tabak, A. (2020). Recycling of an agricultural bio-waste as a novel cellulose aerogel: A green chemistry study. *Journal of Polymers and the Environment*, 28(1), 323-330.

Kaziem, A. E., Yang, L., Lin, Y., Kazem, A. E., Xu, H., and Zhang, Z. X. (2021). Pathogenic Invasion-Responsive Carrier Based on Mesoporous Silica/β-Glucan Nanoparticles for Smart Delivery of Fungicides. *ACS Sustainable Chemistry and Engineering*, 9(27), 9126-9138.

Kaziem, A. E., Yang, L., Lin, Y., Song, Z., Xu, H., and Zhang, Z. (2022). Efficiency of mesoporous silica/carboxymethyl β-glucan as a fungicide nano-delivery system for improving chlorothalonil bioactivity and reduce biotoxicity. *Chemosphere*, 287, 131902.

Kolahi, M.; Kazemi, E. M.; Yazdi, M.; Goldson-Barnaby, A. (2020). Oxidative stress induced by cadmium in lettuce (Lactuca sativa Linn.): Oxidative stress indicators and prediction of their genes. Plant Physiol. Biochem., 146, 71–89.

Kumar, S., Kumar, D., and Dilbaghi, N. (2017). Preparation, characterization, and bio-efficacy evaluation of controlled release carbendazim-loaded polymeric nanoparticles. *Environmental Science and Pollution Research*, 24(1), 926-937.

Kusumavathi, K.; Rautray, S. K.; Sarkar, S.; Dash, S.; Sahoo, T. R.; Swain, S. K.; and Sethi, D. Nano-herbicides a sustainable strategy for weed control. (2024). Plant Nano Biol. 100132.

Lee, J. H.; Kim, K.; Jin, X.; Kim, T. M.; Choi, I. G.; Choi, J. W. Formation of pure nanoparticles with solvent-fractionated lignin polymers and evaluation of their biocompatibility. (2021). Int. J. Biol. Macromol. 183, 660–667.

Lei, C.; and Engeseth, N. J. Comparison of growth characteristics, functional qualities, and texture of hydroponically grown and soil-grown lettuce. (2021). LWT 150, 111931.

Lescano, M. R.; Macagno, J.; Berli, C. L. Model-Based Analysis of Lactuca sativa Root Growth under the Action of Herbicides in Milli-Channel Arrays with In Situ Imaging. (2023). J. Agric. Food Chem. 71(36), 13255–13262.

Li, T., Lü, S., Yan, J., Bai, X., Gao, C., and Liu, M. (2019). An Environment-Friendly Fertilizer Prepared by Layer-by-Layer Self-Assembly for pH-Responsive Nutrient Release. *ACS applied materials and interfaces*, 11(11), 10941-10950.

Li, P., Huang, Y., Fu, C., Jiang, S. X., Peng, W., Jia, Y., and Xu, Z. P. (2021a). Eco-friendly biomolecule-nanomaterial hybrids as next-generation agrochemicals for topical delivery. *EcoMat*.

Li, T., Lü, S., Wang, Z., Huang, M., Yan, J., and Liu, M. (2021b). Lignin-based nanoparticles for recovery and separation of phosphate and reused as renewable magnetic fertilizers. *Science of The Total Environment*, 765, 142745.

Li, Y.; Lin, X.; Xu, G.; Yan, Q.; and Yu, Y. Toxic effects and mechanisms of engineered nanoparticles and nanoplastics on lettuce (Lactuca sativa L.). (2024). Sci. Total Environ. 908, 168421.

Lima, P. H. C. D.; Antunes, D. R.; Forini, M. M. D. L.; Pontes, M. D. S.; Mattos, B. D.; and Grillo, R. Recent advances on lignocellulosic-based nanopesticides for agricultural applications. (2021). Front. Nanotechnol. 3, 809329.

Liu, Y.; Jia, L.; Xiao, Y.; Wang, H.; Luo, X.; Wang, S.; Yin, Q. Solubility Measurement and Thermodynamic Properties of Atrazine in 13 Pure Solvents from 283.15 to 323.15 K. (2024). J. Chem. Eng. Data 70(1), 607–618.

Liu, M.; Xiang, B.; Li, H.; He, X.; Li, H.; Du, K.; Li, X. A sustainable Poly (deep eutectic solvents) based molecular imprinting strategy with experimental and theoretical elucidation: Application for removal of atrazine in agricultural wastewater. (2025). Sep. Purif. Technol. 353, 128637.

Livak, K. J.; and Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. (2001). Methods 25(4), 402–408.

Lizundia, E., Sipponen, M. H., Greca, L. G., Balakshin, M., Tardy, B. L., and Rojas, O. J. and Puglia, D. (2021). Multifunctional lignin-based nanocomposites and nanohybrids. *Green Chemistry*, 23, 6698-6760.

López-Cabeza, R., Kah, M., Grillo, R., Koutný, M., Salač, J., Bílková, Z., and Hofman, J. (2022). Tebuconazole and terbuthylazine encapsulated in nanocarriers: preparation, characterization and release kinetics. Environmental Science: Nano, 9(4), 1427-1438.

Lourencon, T. V., Greca, L. G., Tarasov, D., Borrega, M., Tamminen, T., Rojas, O. J., and Balakshin, M. Y. (2019). Lignin-first integrated hydrothermal treatment (HTT) and synthesis of low-cost biorefinery particles. *ACS Sustainable Chemistry and Engineering*, 8(2), 1230-1239.

Lu, X.; Zhang, Y.; Chen, J.; Zhang, D.; Dong, C.; Zhiqiang, P.; Xia, T. Preparation of tailored lignin nanospheres with specific size and tunable structure by self-assembly of lignin from corn straw. (2021). Ind. Crops Prod. 172, 113993.

Ma, M., Dai, L., Xu, J., Liu, Z., and Ni, Y. (2020). A simple and effective approach to fabricate lignin nanoparticles with tunable sizes based on lignin fractionation. *Green Chemistry*, 22, 2011-2017.

Ma, Y.; Remón, J.; Luo, L.; Ding, W.; Jiang, Z.; and Shi, B. (2025).Natural-Based UV-Shielding Additives to Protect Photosensitive Pesticides: Production of Nanoparticles from the Co-Self-Assembly of Lignin and Tannin. Nano Today 60, 102550.

Machado, T. O., Beckers, S. J., Fischer, J., Muller, B., Sayer, C., Araújo, P.H.H., (2020) Landfester, K. and Wurm, R. F. Bio-based lignin nanocarriers loaded with fungicides as a versatile platform for drug delivery in plants. *Biomacromolecules*, 21, 2755−2763.

Machado, T. O., Beckers, S. J., Fischer, J., Sayer, C., de Araújo, P. H. H., Landfester, K., and Wurm, F. R. (2021). Cellulose nanocarriers via miniemulsion allow Pathogen-Specific agrochemical delivery. *Journal of colloid and interface science*, 601, 678-688.

Majid, M.; Khan, J. N.; Shah, Q. M. A.; Masoodi, K. Z.; Afroza, B.; and Parvaze, S. (2021). Evaluation of Hydroponic Systems for the Cultivation of Lettuce (Lactuca sativa L.; var. Longifolia) and Comparison with Protected Soil-Based Cultivation. Agric. Water Manag. 245, 106572.

Mattos, B. D., and Magalhães, W. L. (2016). Biogenic nanosilica blended by nanofibrillated cellulose as support for slow-release of tebuconazole. *Journal of Nanoparticle Research*, 18(9), 1-10.

Mattos, B. D., Greca, L. G., Tardy, B. L., Magalhães, W. L., and Rojas, O. J. (2018). Green formation of robust supraparticles for cargo protection and hazards control in natural environments. *Small*, 14(29), 1801256.

Mattos, B. D., Tardy, B. L., Magalhães, W. L., and Rojas, O. J. (2017). Controlled release for crop and wood protection: Recent progress toward sustainable and safe nanostructured biocidal systems. *Journal of Controlled Release,* 262, 139-150.

Mirza, L. A.; Salih, R. O.; Abdullah, N. R. Calcite nano-structure as a novel drug carrier for 5-Fluorouracil chemotherapy agent: A computational study using DFT and AIMD. (2025). Computational and Theoretical Chemistry 1248, 115181.

Moreno, A.; Sipponen, M. H. Overcoming Challenges of Lignin Nanoparticles: Expanding Opportunities for Scalable and Multifunctional Nanomaterials. (2024). Acc. Chem. Res. 57(14), 1918–1930.

Munhoz-Garcia, G. V.; Takeshita, V.; de Oliveira, J. L.; Dalla Vecchia, B.; Nalin, D.; Pinácio, C. D. W.; Fraceto, L. F. (2025). Nanobased Natural Polymers as a Carrier System for Glyphosate: An Interesting Approach Aimed at Sustainable Agriculture. J. Agric. Food Chem.

Naidu, D. S., Hlangothi, S. P., and John, M. J. (2018). Bio-based products from xylan: A review. *Carbohydrate polymers*, 179, 28-41.

Neto, F. A. N.; de Freitas Souza, M.; Blat, N. R.; da Silva, F. D.; Fernandes, B. C. C.; das Chagas, P. S. F.; Silva, D. V. (2024). Sensitivity and Antioxidant Response of Forest Species Seedlings to Atrazine under Simulated Conditions of Subsurface Water Contamination. Chemosphere, 360, 142411.

Ng, S. W.; Rosli, N. S. M.; Razali, R. B. R.; Abdullah, R.; and Yaacob, J. S. From Waste to Wealth: Significant Potential of Oil Palm Kernel-Derived Biochar as Alternative Substrate for Production of Red Lettuce (Lactuca sativa L.) with High

Bioactive Metabolites Content in NFT Hydroponic System. (2023). Sustain. Chem. Pharm. 34, 101167.

Niu, N. N.; Zhao, W. J.; Xiao, B. L.; Liang, Y. C.; Meng, X.; Song, X. Y.; Moosavi-Movahedi, A. A. (2021). Quench Treatment Cytochrome c: Transformation from a Classical Redox Protein to a Peroxidase Like Enzyme. J. Iran. Chem. Soc., 1–11.

Okolie, J. A., Nanda, S., Dalai, A. K., and Kozinski, J. A. (2021). Chemistry and specialty industrial applications of lignocellulosic biomass. *Waste and Biomass Valorization*, 12(5), 2145-2169.

Oliveira, H. C.; Stolf-Moreira, R.; Martinez, C. B. R.; Grillo, R.; de Jesus, M. B.; and Fraceto, L. F. Nanoencapsulation Enhances the Post-Emergence Herbicidal Activity of Atrazine against Mustard Plants. (2015). PLoS One 10(7), e0132971.

Österberg, M.; Sipponen, M. H.; Mattos, B. D.; and Rojas, O. J. Spherical Lignin Particles: A Review on Their Sustainability and Applications. (2020). Green Chem. 22(9), 2712–2733.

Papadopoulos, A. N., Bikiaris, D. N., Mitropoulos, A. C., and Kyzas, G. Z. (2019). Nanomaterials and chemical modifications for enhanced key wood properties: A review. *Nanomaterials*, 9(4), 607.

Parkinson, S. J.; Tungsirisurp, S.; Joshi, C.; Richmond, B. L.; Gifford, M. L.; Sikder, A.; Napier, R. M. Polymer Nanoparticles Pass the Plant Interface. (2022). Nat. Commun. 13(1), 7385.

Parven, A., Meftaul, I. M., Venkateswarlu, K., and Megharaj, M. (2024). Herbicides in modern sustainable agriculture: Environmental fate, ecological implications, and human health concerns. International Journal of Environmental Science and Technology, 1-22.

Pearson, R. G. The principle of maximum hardness. (1993). Accounts of Chemical Research 26(5), 250-255.

Peil, S., Beckers, S. J., Fischer, J., and Wurm, F. R. (2020). Biodegradable, lignin-based encapsulation enables delivery of Trichoderma reesei with programmed enzymatic release against grapevine trunk diseases. *Materials Today Bio*, 7, 100061.

Peng, R., Yang, D., Qiu, X., Qin, Y., and Zhou, M. (2020). Preparation of self-dispersed lignin-based drug-loaded material and its application in avermectin nano-formulation. *International journal of biological macromolecules*, 151, 421-427.

Peng, Q.; Ye, L.; Wen, N.; Chen, H.; Zhu, Y.; Niu, H.; Huang, Y. Nitrogen Vacancy-Modified g-C3N4 Nanosheets Controlled by Deep Eutectic Solvents for Highly Efficient Photocatalytic Atrazine Degradation: Non-Radical Dominated Holes Oxidation. (2025). Sep. Purif. Technol. 354, 128879.

Pereira, A. E.; Grillo, R.; Mello, N. F.; Rosa, A. H.; and Fraceto, L. F. Application of Poly(ε-caprolactone) Nanoparticles Containing Atrazine Herbicide as an Alternative Technique to Control Weeds and Reduce Damage to the Environment. (2014). J. Hazard. Mater. 268, 207–215.

Pérez, D. J.; Doucette, W. J.; and Moore, M. T. Atrazine Uptake, Translocation, Bioaccumulation and Biodegradation in Cattail (Typha latifolia) as a Function of Exposure Time. (2022). Chemosphere 287, 132104.

Perotti, V. E.; Larran, A. S.; Palmieri, V. E.; Martinatto, A. K.; and Permingeat, H. R. (2020). Herbicide Resistant Weeds: A Call to Integrate Conventional Agricultural Practices, Molecular Biology Knowledge and New Technologies. *Plant Sci. 290*, 110255.

Pontes, M. S., Antunes, D. R., Oliveira, I. P., Forini, M. M., Santos, J. S., Arruda, G. J., and Grillo, R. (2021). Chitosan/tripolyphosphate nanoformulation carrying paraquat: insights on its enhanced herbicidal activity. *Environmental Science: Nano*, 8(5), 1336-1351.

Preisler, A. C.; Carvalho, L. B.; Saraiva-Santos, T.; Verri Jr, W. A.; Mayer, J. L. S.; Fraceto, L. F.; and Oliveira, H. C. (2022). Interaction of Nanoatrazine and Target Organism: Evaluation of Fate and Photosystem II Inhibition in Hydroponically Grown Mustard (Brassica juncea) Plants.. J. Agric. Food Chem. 70(25), 7644–7652.

Qi, Y.; Li, J.; Fu, G.; Zhao, C.; Guan, X.; Yan, B.; and Ren, M. (2018). Effects of Sublethal Herbicides on Offspring Germination and Seedling Growth: Redroot Pigweed (Amaranthus retroflexus) vs. Velvetleaf (Abutilon theophrasti). Sci. Total Environ., 645, 543–549.

Queiroz, A. M.; Pontes, M. S.; Scherer, M. D.; Graciano, D. E.; Caires, A. R.; and Oliveira, S. L. Root Length as a Bioindicator of Pesticide Concentration in Irrigation Water. (2024). Environ. Sci. Pollut. Res., 1–8.

Ramel, F.; Sulmon, C.; Bogard, M.; Couée, I.; and Gouesbet, G. (2009). Differential Patterns of Reactive Oxygen Species and Antioxidative Mechanisms during Atrazine Injury and Sucrose-Induced Tolerance in Arabidopsis thaliana Plantlets. BMC Plant Biol., 9, 1–18.

Rashidipour, M., Maleki, A., Kordi, S., Birjandi, M., Pajouhi, N., Mohammadi, E., and Davari, B. (2019). Pectin/chitosan/tripolyphosphate nanoparticles: efficient carriers for reducing soil sorption, cytotoxicity, and mutagenicity of paraquat and enhancing its herbicide activity. *Journal of agricultural and food chemistry*, 67(20), 5736-5745.

Rashidipour, M., Rasoulian, B., Maleki, A., Davari, B., Pajouhi, N., and Mohammadi, E. (2021). Pectin/chitosan/tripolyphosphate encapsulation protects the rat lung from fibrosis and apoptosis induced by paraquat inhalation. *Pesticide Biochemistry and Physiology*, 178, 104919.

Rawashdeh, R. Y.; Harb, A. M.; and AlHasan, A. M. Biological Interaction Levels of Zinc Oxide Nanoparticles; Lettuce Seeds as Case Study. (2020). Heliyon 6(5).

Restu, W. K.; Aryana, N.; Septiyanti, M.; Triwulandari, E.; Sampora, Y.; Devy, Y. A.; and Ogino, C. Development of a Lignin-Based Carrier-Citronella Oil Nanoemulsion and Its Utilization as an Herbicide against Specific Weed Species. (2024). Chem. Biodiversity 21(9), e202400861.

Restu, W. K.; Septiyanti, M.; Triwulandari, E.; Aryana, N.; Sampora, Y.; Devy, Y. A.; and Ogino, C. The Preparation of Nanoemulsion Formulation from Pelargonic Acid in Lignin Carrier and Its Application to Weeds. (2023). J. Inst. Eng. (India): Ser. E 104(2), 297–303.

Ribeiro, C.; Stitt, M.; Hotta, C. T. How Stress Affects Your Budget—Stress Impacts on Starch Metabolism. (2022). Front. Plant Sci. 13, 774060.

Ritger, P. L.; and Peppas, N. A. A Simple Equation for Description of Solute Release I. Fickian and Non-Fickian Release from Non-Swellable Devices in the Form of Slabs, Spheres, Cylinders or Discs. (1987). J. Control. Release 5(1), 23–36.

Rodrigues, J. S.; Takeshita, V.; Campos, E. V.; de Freitas, A. S.; de Lima, V. H.; and Fraceto, L. F. Lignocellulosic Biomass in Nanopesticides: A Path toward Sustainable Agriculture. (2024). ACS Sustainable Chem. Eng. 12(27), 10045–10067.

Rop, K., Mbui, D., Karuku, G. N., Michira, I., and Njomo, N. (2020). Characterization of water hyacinth cellulose-g-poly (ammonium acrylate-co-acrylic acid)/nano-hydroxyapatite polymer hydrogel composite for potential agricultural application. *Results in Chemistry*, 2, 100020.

Rostami, S., Jafari, S., Moeini, Z., Jaskulak, M., Keshtgar, L., Badeenezhad, A. and Dehghani, M. (2021). Current methods and technologies for degradation of atrazine in contaminated soil and water: A review. Environmental Technology and Innovation, 24, 102019.

Saleem, H., and Zaidi, S. J. (2020). Recent developments in the application of nanomaterials in agroecosystems. *Nanomaterials*, 10(12), 2411.

Salinas, F.; Astete, C. E.; Waldvogel, J. H.; Navarro, S.; White, J. C.; Elmer, W.; Sabliov, C. M. (2021). Effects of Engineered Lignin-Graft-PLGA and Zein-Based Nanoparticles on Soybean Health. NanoImpact, 23, 100329.

Santo Pereira, A. D. E.; de Oliveira, J. L.; Savassa, S. M.; Rogerio, C. B.; de Medeiros, G. A.; and Fraceto, L. F. Lignin Nanoparticles: New Insights for a Sustainable Agriculture. (2022). J. Cleaner Prod. 345, 131145.

Sathishkumar, P.; Li, Z.; Govindan, R.; Jayakumar, R.; Wang, C.; Gu, F. L. Zinc Oxide-Quercetin Nanocomposite as a Smart Nano-Drug Delivery System: Molecular-Level Interaction Studies. (2021). Appl. Surf. Sci. 536, 147741.

Schiavi, D., Balbi, R., Giovagnoli, S., Camaioni, E., Botticella, E., Sestili, F., and Balestra, G. M. (2021). A Green Nanostructured Pesticide to Control Tomato Bacterial Speck Disease. *Nanomaterials*, 11(7), 1852.

Shahi, N., Wang, P., Adhikari, S., Min, B., and Rangari, V. K. (2021). Biopolymers Fractionation and Synthesis of Nanocellulose/Silica Nanoparticles from Agricultural Byproducts. ACS Sustainable *Chemistry and Engineering*, 9(18), 6284-6295.

Shahid, M.; Shafi, Z.; Ilyas, T.; Singh, U. B.; Pichtel, J. Crosstalk between Phytohormones and Pesticides: Insights into Unravelling the Crucial Roles of Plant Growth Regulators in Improving Crop Resilience to Pesticide Stress. (2024). Sci. Hortic. 338, 113663.

Shakiba, S.; Astete, C. E.; Paudel, S.; Sabliov, C. M.; Rodrigues, D. F.; Louie, S. M. Emerging investigator series: polymeric nanocarriers for agricultural applications: synthesis, characterization, and environmental and biological interactions. (2020). Environ. Sci.: Nano 7(1), 37–67.

Sharif, S. N. M., Hashim, N., Isa, I. M., Bakar, S. A., Saidin, M. I., Ahmad, M. S., and Zainul, R. (2021). Polymeric Nanocomposite-Based Herbicide of Carboxymethyl Cellulose Coated-Zinc/Aluminium Layered Double Hydroxide-Quinclorac: A Controlled Release Purpose for Agrochemicals. *Journal of Polymers and the Environment*, 29(6), 1817-1834.

Sharifan, H.; Ma, X.; Moore, J. M.; Habib, M. R.; and Evans, C. Zinc Oxide Nanoparticles Alleviated the Bioavailability of Cadmium and Lead and Changed the Uptake of Iron in Hydroponically Grown Lettuce (Lactuca sativa L. var. Longifolia). (2019). ACS Sustainable Chem. Eng. 7(19), 16401.

Sharma, R., Bajpai, J., Bajpai, A. K., Acharya, S., Kumar, B., and Singh, R. K. (2017). Assessment of water retention performance of pectin-based nanocarriers for controlled irrigation in agriculture. *Agricultural Research*, 6(2), 139-149.

Sharma, S.; Kachhia, P.; Shukla, V.; Patel, A.; Rathod, V.; Narra, M. Valorization of lignin into nanoparticles and nanogel: Characterization and application. (2022). Bioresour. Technol. Rep. 18, 101041.

Shatilov, M. V.; Razin, A. F.; and Ivanova, M. I. Analysis of the world lettuce market. In IOP Conf. Ser.: Earth Environ. Sci.; IOP Publishing, 2019; Vol. 395, No. 1, p 012053.

Shatkin, J. A., and Kim, B. (2015). Cellulose nanomaterials: life cycle risk assessment, and environmental health and safety roadmap. *Environmental Science: Nano*, 2(5), 477-499.

Sherard, M. M.; Dang, Q. M.; Reiff, S. C.; Simpson, J. H.; Leopold, M. C. On-site detection of neonicotinoid pesticides using functionalized gold nanoparticles and halogen bonding. (2023). ACS Appl. Nano Mater. 6(10), 8367–8381.

Shrestha, S., Kognou, A. L. M., Zhang, J., and Qin, W. (2021). Different facets of lignocellulosic biomass including pectin and its perspectives. Waste and Biomass Valorization, 12(9), 4805-4823.

Shu, F.; Jiang, B.; Yuan, Y.; Li, M.; Wu, W.; Jin, Y.; and Xiao, H. Biological activities and emerging roles of lignin and lignin-based products—A review. (2021). Biomacromolecules 22(12), 4905–4918.

Sikder, A., Pearce, A. K., Parkinson, S. J., Napier, R., and O'Reilly, R. K. (2021). Recent trends in advanced polymer materials in agriculture related applications. *ACS Applied Polymer Materials*, 3(3), 1203-1217.

Simoni, S.; Castellacci, M.; Usai, G.; Rogo, U.; Mascagni, F.; Giordani, T.; Vangelisti, A. (2024). Genome-Wide Characterization of Class III Peroxidases and Their Expression Profile during Mycorrhizal Symbiosis and Phosphorus Deprivation in Lettuce (Lactuca sativa L.). Horticulturae, 10(11), 1170.

Singh, H., Sharma, A., Bhardwaj, S. K., Arya, S. K., Bhardwaj, N., and Khatri, M. (2021). Recent advances in the applications of nano-agrochemicals for sustainable agricultural development. *Environmental Science: Processes and Impacts*, 23(2), 213-239.

Sipponen, M. H., Rojas, O. J., Pihlajaniemi, V., Lintinen, K., and Österberg, M. (2017). Calcium chelation of lignin from pulping spent liquor for water-resistant slow-release urea fertilizer systems. *ACS Sustainable Chemistry and Engineering*, *5*(1), 1054-1061.

Siqueira, J. G. W., Rodrigues, C., de Souza Vandenberghe, L. P., Woiciechowski, A. L., and Soccol, C. R. (2020). Current advances in on-site cellulase production and application on lignocellulosic biomass conversion to biofuels: a review. *Biomass and Bioenergy*, 132, 105419.

Sobiecka, E.; Mroczkowska, M.; Olejnik, T. P. (2022). The Enzymatic Antioxidants Activities Changes in Water Plants Tissues Exposed to Chlorpyrifos Stress. Antioxidants, 11(11), 2104.

Song, L.; Cheng, H.; Liu, C.; Ji, R.; Yao, S.; Cao, H.; and Song, Y. Oyster Shell Facilitates the Green Production of Nitrogen-Doped Porous Biochar from Macroalgae: A Case Study for Removing Atrazine from Water. (2024). Biochar 6(1), 76.

Sousa, G. F.; Gomes, D. G.; Campos, E. V.; Oliveira, J. L.; Fraceto, L. F.; Stolf-Moreira, R.; Oliveira, H. C. (2018). Post-Emergence Herbicidal Activity of Nanoatrazine against Susceptible Weeds. *Front. Environ. Sci*, *6*, 12.

Sousa, B. T.; Carvalho, L. B.; Preisler, A. C.; Saraiva-Santos, T.; Oliveira, J. L.; Verri Jr, W. A.; and Oliveira, H. Chitosan Coating as a Strategy to Increase Postemergent Herbicidal Efficiency and Alter the Interaction of Nanoatrazine with Bidens pilosa Plants. (2024). ACS Appl. Mater. Interfaces.

Stanisz, M.; Collins, M. N.; and Jesionowski, T. Recent progress in biomedical and biotechnological applications of lignin-based spherical nano- and microstructures: a comprehensive review. (2022). Mater. Today Chem. 26, 101198.

Stloukal, P.; Kucharczyk, P.; Sedlarik, V.; Bazant, P.; Koutny, M. Low molecular weight poly(lactic acid) microparticles for controlled release of the herbicide metazachlor: preparation, morphology, and release kinetics. (2012). J. Agric. Food Chem. 60(16), 4111–4119.

Su, S., Chen, L., Hao, L., Chen, H., Zhou, X., and Zhou, H. (2021a). Fluorinated sodium carboxymethyl cellulose nanoparticles as carrier for improving adhesion and sustaining release of AVM. *Journal of Macromolecular Science*, Part A, 58(4), 219-231.

Su, S., Chen, L., Hao, L., Chen, H., Zhou, X., and Zhou, H. (2021c). Preparation of p-amino salicylic acid-modified polysuccinimide as water-based nanocarriers for enhancing pesticide stability and insecticidal activity. *Colloids and Surfaces B: Biointerfaces*, 207, 111990.

Su, Y., Chen, L., Yang, F., and Cheung, P. C. (2021b). Beta-d-glucan-based drug delivery system and its potential application in targeting tumor associated macrophages. *Carbohydrate polymers*, 253, 117258.

Sun, H., Erdman III, W., Yuan, Y., Mohamed, M. A., Xie, R., Wang, Y., and Cheng, C. (2020). Crosslinked polymer nanocapsules for therapeutic, diagnostic, and theranostic applications. Wiley Interdisciplinary Reviews: *Nanomedicine and Nanobiotechnology*, 12(6), e1653.

Swapnil, P.; Meena, M.; Singh, S. K.; Dhuldhaj, U. P.; Marwal, A. (2021). Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. Curr. Plant Biol., 26, 100203.

Taban, A.; Saharkhiz, M. J.; Khorram, M. (2020). Formulation and assessment of nano-encapsulated bioherbicides based on biopolymers and essential oil. Ind. Crops Prod., 149, 112348.

Takeshita, V.; Oliveira, F. F.; Garcia, A.; Zuverza-Mena, N.; Tamez, C.; Cardoso, B. C.; and White, J. C. Delivering metribuzin from biodegradable nanocarriers: assessing herbicidal effects for soybean plant protection and weed control. (2025). Environ. Sci.: Nano.

Tardy, B. L., Mattos, B. D., Otoni, C. G., Beaumont, M., Majoinen, J., Kämäräinen, T., and Rojas, O. J. (2021). Deconstruction and Reassembly of Renewable Polymers and Biocolloids into Next Generation Structured Materials. *Chemical Reviews*.

Teo, H. L., and Wahab, R. A. (2020). Towards an eco-friendly deconstruction of agro-industrial biomass and preparation of renewable cellulose nanomaterials: A review. *International journal of biological macromolecules*.

Thakur, N., and Yadav, A. N. (2025). Nanotechnology in Agriculture: A Review on Precision Farming and Sustainable Crop Production. BioNanoScience, 15(2), 243.

Trevisan, H.; and Rezende, C. A. Pure, stable and highly antioxidant lignin nanoparticles from elephant grass. (2020). Ind. Crops Prod. 145, 112105.

Tripathi, D.; Singh, M.; Pandey-Rai, S. (2022). Crosstalk of nanoparticles and phytohormones regulate plant growth and metabolism under abiotic and biotic stress. Plant Stress, 6, 100107.

Ur Rahim, H., Qaswar, M., Uddin, M., Giannini, C., Herrera, M. L., and Rea, G. (2021). Nano-Enable Materials Promoting Sustainability and Resilience in Modern Agriculture. Nanomaterials, 11(8), 2068.

Usman, M., Farooq, M., Wakeel, A., Nawaz, A., Cheema, S. A., ur Rehman, H., and Sanaullah, M. (2020). Nanotechnology in agriculture: Current status, challenges and future opportunities. *Science of the Total Environment*, 721, 137778.

Vieira, L. A.; Alves, R. D.; Menezes-Silva, P. E.; Mendonça, M. A.; Silva, M. L.; Silva, M. C.; Farnese, F. S. Water contamination with atrazine: is nitric oxide able to improve Pistia stratiotes phytoremediation capacity? (2021). Environ. Pollut. 272, 115971.

Vinzant, K., Rashid, M., Clouse, D. E., Ghosh, P., Quadir, M., Davis, V. A., and Khodakovskaya, M. V. (2025). From Plants to Plants: Plant-Derived Biological Polymers as Sustainable and Safe Nanocarriers for Direct Delivery of DNA to Plant Cells. Nano Letters.

Wang, D.; Saleh, N. B.; Byro, A.; Zepp, R.; Sahle-Demessie, E.; Luxton, T. P.; Su, C. (2022). Nano-enabled pesticides for sustainable agriculture and global food security. Nat. Nanotechnol., 17(4), 347–360.

Wang, J.; Ma, Y.; Huang, X.; Song, L.; Li, N.; Cheng, Y.; and Hai, D. Changes in physio-biochemical metabolism, phenolics and antioxidant capacity of different Chinese pea varieties during germination. (2023). Int. J. Food Sci. Technol. 58(1), 167–180.

Wang, N.; Cheng, X.; Li, N.; Wang, H.; and Chen, H. Nanocarriers and their loading strategies. (2019). Adv. Healthcare Mater. 8(6), 1801002.

Wang, Q.; Wang, H.; Chen, H.; Meng, Z.; Kong, F.; Liu, Y.; and Jiang, W. Efficient synthesis of highly hydrophobic lignin nanoparticles and their application as nano fillers for multifunctional composite membranes. (2024). Int. J. Biol. Macromol. 282, 136869.

Wang, H.; Liu, M.; Tan, S.; Li, X.; Huang, M.; Hao, X.; and Peng, F. Porous lignin nanospheres as microscopic air purifiers for antioxidant-configurable cigarette filters. (2025a). Adv. Funct. Mater. 2420731.

Wang, Y.; Chen, Y.; Wang, L.; Mo, Y.; Lin, X.; Gao, S.; Chen, M. Efficient removal of atrazine in wastewater by washed peanut shells biochar: Adsorption behavior and biodegradation. (2025b). Process Biochem.

Wang, Y.; Ma, X.; Shang, W.; Luo, H.; Wang, L.; Sun, K.; Zhang, T. (2025c). Comparative transcriptome analysis reveals differential mechanisms of soft rot resistance in lettuce grown under white and blue light. Food Energy Secur., 14(1), e70038.

Weng, Y.; Bai, X.; Kang, M.; Ji, Y.; Wang, H.; Liu, Y. (2025). Detoxification strategy of titanium oxide nanoparticles driving endogenous molecules metabolism to modulate atrazine conversion in Lactuca sativa L. Environ. Sci. Technol., 59(13), 6440–6451.

Wijaya, C. J., Ismadji, S., and Gunawan, S. (2021). A Review of Lignocellulosic-Derived Nanoparticles for Drug Delivery Applications: Lignin Nanoparticles, Xylan Nanoparticles, and Cellulose Nanocrystals. *Molecules*, 26(3), 676.

Worrall, E. A., Hamid, A., Mody, K. T., Mitter, N., and Pappu, H. R. (2018). Nanotechnology for plant disease management. *Agronomy*, 8(12), 285.

Wu, J.; Zhai, Y.; Monikh, F. A.; Arenas-Lago, D.; Grillo, R.; Vijver, M. G.; and Peijnenburg, W. J. The differences between the effects of a nanoformulation and a conventional form of atrazine to lettuce: physiological responses, defense mechanisms, and nutrient displacement. (2021). J. Agric. Food Chem. 69(42), 12527–12540.

Xie, J.; Cao, B.; and Xu, K. Uncovering the dominant role of root lignin accumulation in silicon-induced resistance to drought in tomato. (2024). Int. J. Biol. Macromol. 259, 129075.

Xing, R.; He, J.; Hao, P.; Zhou, W. Graphene oxide-supported nanoscale zero-valent iron composites for the removal of atrazine from aqueous solution. (2020). Colloids and Surfaces A: Physicochemical and Engineering Aspects 589, 124466.

Xiong, F.; Han, Y.; Wang, S.; Li, G.; Qin, T.; Chen, Y.; Chu, F. Preparation and formation mechanism of size-controlled lignin nanospheres by self-assembly. (2017). Ind. Crops Prod. 100, 146–152.

Xu, J.; Luo, X.; Wang, Y.; and Feng, Y. Evaluation of zinc oxide nanoparticles on lettuce (Lactuca sativa L.) growth and soil bacterial community. (2018). Environ. Sci. Pollut. Res. 25, 6026–6035.

Xu, L.; Wang, X.; Shi, H.; Hua, B.; Burken, J. G.; Ma, X.; and Yang, J. J. Uptake of engineered metallic nanoparticles in soil by lettuce in single and binary nanoparticle systems. (2022). ACS Sustainable Chem. Eng. 10(50), 16692–16700.

Yamaguchi, A.; Mimura, N.; Shirai, M.; and Sato, O. Bond cleavage of lignin model compounds into aromatic monomers using supported metal catalysts in supercritical water. (2017). Sci. Rep. 7(1), 46172.

Yao, T.; Feng, K.; Xie, M.; Barros, J.; Tschaplinski, T. J.; Tuskan, G. A.; and Chen, J. G. Phylogenetic occurrence of the phenylpropanoid pathway and lignin biosynthesis in plants. (2021). Front. Plant Sci. 12, 704697.

Ye, L., Han, Y., Wang, X., Lu, X., Qi, X., and Yu, H. (2021). Recent progress in furfural production from hemicellulose and its derivatives: Conversion mechanism, catalytic system, solvent selection. *Molecular Catalysis*, 515, 111899.

Yearla, S. R., and Padmasree, K. (2016). Exploitation of subabul stem lignin as a matrix in controlled release agrochemical nanoformulations: a case study with herbicide diuron. *Environmental Science and Pollution Research*, 23(18), 18085-18098.

Yiamsawas, D., Kangwansupamonkon, W., and Kiatkamjornwong, S. (2021). Lignin-based nanogels for the release of payloads in alkaline conditions. *European Polymer Journal*, 145, 110241.

Yin, J. M., Wang, H. L., Yang, Z. K., Wang, J., Wang, Z., Duan, L. S., and Tan, W. M. (2020). Engineering Lignin Nanomicroparticles for the Antiphotolysis and Controlled Release of the Plant Growth Regulator Abscisic Acid. *Journal of Agricultural and Food Chemistry*, 68(28), 7360-7368.

Yu, M., Sun, C., Xue, Y., Liu, C., Qiu, D., Cui, B., and Zeng, Z. (2019). Tannic acid-based nanopesticides coating with highly improved foliage adhesion to enhance foliar retention. *RSC advances*, *9*(46), 27096-27104. h

Yu, M.; Dong, H.; Liu, K.; Zheng, Y.; Hoffmann, M. R.; Liu, W. (2021). Porous carbon monoliths for electrochemical removal of aqueous herbicides by “one-stop” catalysis of oxygen reduction and H2O2 activation. *J. Hazard. Mater. 414*, 125592.

Zhai, Y.; Abdolahpur Monikh, F.; Wu, J.; Grillo, R.; Arenas-Lago, D.; Darbha, G. K.; Vijver, M. G.; and Peijnenburg, W. J. G. M. Interaction between a nano-formulation of atrazine and rhizosphere bacterial communities: atrazine degradation and bacterial community alterations. (2020). Environ. Sci.: Nano 7, 3372–3384.

Zhang, L., Peng, X., Zhong, L., Chua, W., Xiang, Z., and Sun, R. (2019). Lignocellulosic biomass derived functional materials: synthesis and applications in biomedical engineering. *Current medicinal chemistry*, 26(14), 2456-2474.

Zhang, S., Fu, X., Tong, Z., Liu, G., Meng, S., Yang, Y., and Li, Y. C. (2020). Lignin–Clay Nanohybrid Biocomposite-Based Double-Layer Coating Materials for Controllable-Release Fertilizer. *ACS Sustainable Chemistry and Engineering,* 8(51), 18957-18965.

Zhang, D. X., Du, J., Wang, R., Luo, J., Jing, T. F., Li, B. X., and Hou, Y. (2021a). Core/Shell Dual-Responsive Nanocarriers via Iron-Mineralized Electrostatic Self-Assembly for Precise Pesticide Delivery. *Advanced Functional Materials*, 2102027.

Zhang, J. J.; Yang, H. Metabolism and detoxification of pesticides in plants. (2021b). Sci. Total Environ. 790, 148034.

Zhang, Z.; Marin, C. B.; Lefebvre, M.; Lefebvre, C.; Terrasson, V.; Guénin, E. The preparation of stable spherical alkali lignin nanoparticles with great thermal stability and no cytotoxicity. (2022). Int. J. Biol. Macromol. 222, 1830–1839.

Zhang, Y.; Xu, J.; Li, R.; Ge, Y.; Li, Y.; Li, R. (2023). Plants' response to abiotic stress: Mechanisms and strategies. Int. J. Mol. Sci., 24(13), 10915.

Zhang, H.; Xu, W.; Li, G.; Qu, H.; Ma, C.; Noruzi, E. B.; Li, H. Controlled release system of nanopesticides based on noncovalent interactions. (2024a). ACS Agric. Sci. Technol. 4(9), 851–871.

Zhang, Y.; Li, Z.; Zhang, N.; Chai, X. (2024b). The effects of FR and UVA irradiation timing on multi-omics of purple lettuce in plant factories. Agriculture, 14(11), 2019.

Zhang, L., Xu, L., Zhang, Z., Li, J., Ren, L., Liu, Z., and Chen, Y. (2025). Influence Mechanism of Vermicompost with Different Maturity on Atrazine Catabolism and Bacterial Community. *Toxics*, *13*(1), 30.

Zhao, J.; Lu, C.; Tariq, M.; Xiao, Q.; Zhang, W.; Huang, K.; Liu, Z. (2019). The response and tolerance mechanisms of lettuce (Lactuca sativa L.) exposed to nickel in a spiked soil system. Chemosphere, 222, 399–406.

Zhao, M., Zhou, H., Hao, L., Chen, H., and Zhou, X. (2021a). A high-efficient nano pesticide-fertilizer combination fabricated by amino acid-modified cellulose based carriers. *Pest Management Science*.

Zhao, M., Zhou, H., Hao, L., Chen, H., and Zhou, X. (2021b). Natural rosin modified carboxymethyl cellulose delivery system with lowered toxicity for long-term pest control. *Carbohydrate Polymers*, 259, 117749.

Zhao, Y. W.; Wang, C. K.; Huang, X. Y.; Hu, D. G. (2021c). Anthocyanin stability and degradation in plants. Plant Signal. Behav., 16(12), 1987767.

Zhao, H.; Chen, H.; Zhao, B. (2024). Glycolic acid addition enhances lead uptake and transport by Hydrangea macrophylla (Thunb.) Ser. of different plant ages. Environ. Technol. Innov., 36, 103877.

Zhi, H., Yu, M., Yao, J., Sun, C., Cui, B., Zhao, X., and Zeng, Z. (2020). A facile approach to increasing the foliage retention of pesticides based on coating with a tannic acid/Fe3+ complex. *Coatings*, *10*(4), 359.

Zhou, J.; Hu, Q.; Xiao, X.; Yao, D.; Ge, S.; Ye, J.; Xing, W. (2021). Mechanism of phosphate sensing and signaling revealed by rice SPX1-PHR2 complex structure. Nat. Commun., 12(1), 7040.

Zhu, H., Shen, Y., Cui, J., Li, N., Wang, C., Cui, B., and Cui, H. (2020). Avermectin loaded carboxymethyl cellulose nanoparticles with stimuli-responsive and controlled release properties. Industrial *Crops and Products*, 152, 112497.