



Faculty of Resource Science and Technology

**Pristine and Iodo-Lignin Nanoparticles Prepared via Nanoprecipitation
for Antifungal Applications**

Marshallanore Mhellus anak Amat

**Master of Science
2024**

Pristine and Iodo-Lignin Nanoparticles Prepared via Nanoprecipitation for Antifungal Applications

Marshallanore Mhellus anak Amat

A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Physical Chemistry)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2024

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

..........

Signature

Name: Marshallanore Mhellus anak Amat

Matric No.: 20020346

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date :

ACKNOWLEDGEMENT

First of all, I would like to take this opportunity to express my acknowledgment to my supervisor Prof. Dr. Chin Suk Fun from the Faculty of Resource Science and Technology (FRST), UNIMAS, for encouraging, helping, motivating and guiding me throughout this project research. I am grateful to my supervisor, Prof. Dr. Chin Suk Fun for giving me this opportunity to do this title research and gratitude to Sarawak Timber Development Centre through grant GL/F07/PUSAKA/2021;SaBIDTeC/R&D/2020/002 for financial support. Apart from that, I would like to thank and express my gratitude to Dr. Simon Ngieng for his guidance in my antifungal studies and molecular works. Due to his guidance, I have achieved an abundance of knowledge and experience about the techniques of antifungal screening.

Also, I would like to take this opportunity to thank Mr. Shafri, the assistant science officer who helped me in analysing my samples by using a scanning electron microscope (SEM) and Mdm. Ting helped me in analysing my samples by using a transmission electron microscope (TEM) and light microscope analysis. Besides that, I am grateful to my seniors and lab mate, especially Ho Boon Kui who has guided me through this research and provided me with a lot of useful information. Also, my warmest thanks to family members, Scholastica, and Nurhaziqah for guiding and helping me with my antifungal studies.

Finally, I would like to thank my co-supervisors, Dr. Effendi and Prof. Awang for supporting and guiding me throughout my research studies. Also, I would like to thank the management of the Faculty of Resource Science and Technology (FRST) and Centre for Graduate Studies (CGS) for making it possible for me to complete my research work.

ABSTRACT

In this study, lignin and iodo-lignin nanoparticles were prepared by nanoprecipitation method. Lignin nanoparticles (LNPs) demonstrated a decrease in particle size from 200 nm to 20 nm with an increased volume of water from 10 mL to 50 mL. Meanwhile, the obtained lignin nanoparticles are functionalised with iodine by laccase catalysed iodination. Iodo-lignin nanoparticles (I-LNPs) shows decreased nanosized from 350 nm to 40 nm with decreased volume of water (50 mL to 10 mL) after observed under SEM and TEM analysis. Further, antifungal activity was evaluated on LNPs and I-LNPs at different particles size (20 nm to 350 nm) and different concentrations (3 mM to 12 mM) against *Fusarium equiseti*, *Cunninghamella* sp., *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma piluliferum* and *Penicillium chermesinum*. The maximum antifungal activity was achieved by decreasing nanosized and increasing the concentration of LNPs and I-LNPs. Mycelium inhibition of I-LNPs were greatly decreased about 65% to 98 % with decreasing nanosized and increasing concentration against the tested fungi. The best antifungal results were achieved using I-LNPs size at 40 nm combined with 12 mM concentration. Meanwhile, LNPs demonstrated lower inhibition about 20% to 75%. Similarly, the best result for LNPs was achieved using the smallest nanosized at 20 nm combined with 12 mM concentration. The potential application of LNPs and I-LNPs were further tested on bamboo by impregnation of nanoparticles via laccase catalysed reaction. The molecular results revealed the isolated fungi as *Cunninghamella* sp., *Pleosporales* sp. and *P. sumatrense*. Then, these fungi were tested against LNPs and I-LNPs. Interestingly, I-LNPs shows promising results about 80% to 90% of mould resistance against *Cunninghamella* sp., *Pleosporales* sp., and *P. sumatrense*. Further decay resistance test of I-LNPs treated bamboo shows excellent results with less than 5% of mass loss. After leaching, I-LNPs shows good decay resistance with

less than 8 % of mass loss after 90 days of incubation. This work has revealed that I-LNPs and LNPs can be a potential candidate as antifungal agents against plant pathogenic fungi.

Keywords: Lignin nanoparticles, iodo-lignin nanoparticles, antifungal activity, mould resistance, decay resistance

Nanopartikel Asli dan Iodo-lignin yang Disediakan melalui Pemendakan untuk Aplikasi Antikulat

ABSTRAK

Dalam kajian ini, nanopartikel lignin dan iodo-lignin telah disediakan dengan kaedah pemendakan nano. Nanopartikel lignin (NPL) menunjukkan penurunan saiz zarah daripada 200 nm kepada 20 nm dengan peningkatan isipadu air daripada 10mL hingga 50 mL. Sementara itu, nanopartikel lignin yang diperoleh difungsikan dengan iodine melalui pengionan bermangkin lakase. Nanopartikel iodo-lignin (NPIL) menunjukkan saiz zarah yang berkurangan daripada 300 nm kepada 40 nm dengan penurunan isipadu air (50 mL hingga 10 mL) selepas diperhatikan di bawah analisis SEM dan TEM. Selanjutnya, aktiviti antikulat dinilai pada NPL dan NPIL pada saiz zarah yang berbeza dan kepekatan berbeza (3 mM hingga 12 mM) terhadap *Fusarium equiseti*, *Cunninghamella* sp., *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma piluliferum* dan *Penicillium chermesinum*. Aktiviti antikulat maksimum dicapai dengan mengurangkan saiz nano dan meningkatkan kepekatan NPL dan NPIL. Perencatan miselium NPIL telah berkurangan dengan banyak iaitu kira-kira 65% hingga 98% dengan pengurangan saiz nano dan peningkatan kepekatan terhadap kulat yang diuji. Keputusan antikulat terbaik dicapai menggunakan saiz NPIL pada 40 nm digabungkan dengan kepekatan 12 mM. Sementara itu, NPL menunjukkan perencatan yang lebih rendah kira-kira 20% hingga 75%. Begitu juga, keputusan terbaik untuk NPL dicapai menggunakan saiz NPL terkecil pada 20 nm digabungkan dengan kepekatan 12 mM. Potensi aplikasi NPL dan NPIL telah diuji selanjutnya pada buluh dengan impregnasi nanopartikel melalui tindak balas pemangkin lakase. Kulat yang dikenal pasti secara molekular daripada buluh telah dikenal pasti sebagai *Cunninghamella* sp. dan *Pleosporales* sp. dan *P.*

sumatrense. Kemudian, kulat ini diuji terhadap NPL dan NPIL. Menariknya, NPIL menunjukkan hasil yang menjanjikan dengan kira-kira 80% hingga 90% rintangan kulat terhadap Cunninghamella sp., Pleosporales sp. dan P. sumatrense. Ujian rintangan pereputan selanjutnya menunjukkan keputusan yang sangat baik dengan kurang daripada 5% kehilangan jisim untuk NPIL yang tidak larut lesap. Selepas larut lesap, NPIL menunjukkan rintangan pereputan yang baik dengan kurang daripada 8% kehilangan jisim selepas 90 hari pengesanan. Kerja ini telah mendedahkan bahawa NPIL dan NPL boleh menjadi calon berpotensi sebagai agen antikulat terhadap kulat tumbuhan yang patogenik.

Kata kunci: Nanopartikel lignin, nanopartikel iodo-lignin, aktiviti antikulat, rintangan kulat, rintangan pereputan

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
ABSTRAK	v
TABLE OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xiv
CHAPTER 1 INTRODUCTION	1
1.1 Study Background	1
1.2 Problem Statement	3
1.3 Objectives	4
CHAPTER 2 LITERATURE REVIEW	5
2.1 Overview of Lignin	5
2.2 Synthesis of Lignin Nanoparticles	6
2.2.1 Mechanical Method	6
2.2.2 Nanoprecipitation by Solvent Shifting Method	7

2.3	Functionalisation of Lignin Nanoparticles	10
2.4	Iodine-based Compound as Antifungal Agent	11
2.5	Plant Pathogenic Fungi	13
2.6	Background of Bamboo	15
2.6.1	<i>Dendrocalamus asper</i>	16
2.7	Bamboo Treatment Method	17
2.7.1	Harvesting Practises	17
2.7.2	Nanoparticles Impregnation	18
2.7.3	Chemical Preservation	20
2.8	Fungi Infecting Bamboo	21
CHAPTER 3 MATERIALS AND METHODS		23
3.1	Materials	23
3.2	Preparation of Lignin Nanoparticles	23
3.3	Preparation of Iodine Functionalised Lignin Nanoparticles	24
3.4	Characterisation of Samples	24
3.5	Antifungal Assay	25
3.6	Preparation of Bamboo	26
3.7	Isolation of Fungi from Bamboo	27
3.8	Molecular Identification of Fungi	28
3.9	Mould Test and Characterisation	28

3.10	Mass Loss of Bamboo	30
CHAPTER 4 RESULTS AND DISCUSSION		31
4.1	Characterisation of LNPs via SEM and TEM Analyses	31
4.2	UV-vis Analysis of I-LNPs	34
4.3	FT-IR Spectroscopy	35
4.4	Characterisation of I-LNPs via SEM and TEM Analyses	37
4.5	EDX Analysis of LNPs and I-LNPs	40
4.6	Dispersion stability of LNPs and I-LNPs	41
4.7	Antifungal Activity	43
4.8	Surface Morphology of Bamboo	51
4.8.1	SEM Analysis of Bamboo	51
4.8.2	EDX Analysis of Bamboo	53
4.9	Identification of Fungi	54
4.9.1	Macroscopic and Macroscopic Identification	54
4.9.2	Molecular Identification	56
4.10	Antifungal Activity of Bamboo	57
4.10.1	Mould Resistance	57
4.10.2	Decay Resistance	59
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS		61
5.1	Conclusion	61

5.2	Recommendations	61
	REFERENCES	63
	APPENDICES	89

LIST OF TABLES

	Page
Table 3.1: Ranking System for Specimen Mould Damage (AWPA E-10, 2020)	29
Table 4.1: Antifungal Activity of LNPs Against Various Plant Pathogenic Fungi (%) (n = 3)	45
Table 4.2: Antifungal Activity of I-LNPs Against Various Plant Pathogenic Fungi (%) (n = 3)	49
Table 4.3: Mass Loss of Bamboo After 12 weeks of Incubations (n = 3)	60

LIST OF FIGURES

	Page
Figure 2.1: Structure of Lignin	5
Figure 2.2: The Formation of Lignin Nanoparticles via Self-Assembly (Ago et al., 2017)	8
Figure 2.3: Laccase Catalysis of Iodo-Lignin (Sdahl et al., 2019)	11
Figure 2.4: Biocidal Mechanisms of Iodine on Cell Wall of Fungi (Navarro et al., 2021)	12
Figure 2.5: The Image of <i>Dendrocalamus asper</i>	16
Figure 2.6: The Image of <i>Dendrocalamus asper</i> Infected by Plant Pathogenic Fungi	22
Figure 3.1: Preparation of Bamboo by Laccase-Catalysed Oxidation of LNPs and I-LNPs	26
Figure 4.1: Mean Particle Sizes of LNPs With Different Volumes of Water (n = 3)	31
Figure 4.2: SEM Micrographs of LNPs After Final Addition of (a) 10 mL, (b) 20 mL, (c) 30 mL, (d) 40 mL and (e) 50 mL of Water	32
Figure 4.3: TEM Micrographs of LNPs After Final Addition of (a) 10 mL, (b) 20 mL, (c) 30 mL, (d) 40 mL, (e) 50 mL of Water and (f) Particle Sizes Distribution of LNPs	33
Figure 4.4: UV-vis Spectra of LNPs and I-LNPs	34
Figure 4.5: FT-IR Spectroscopy of (a) Lignin Nanoparticles (LNPs) and (b) Iodo-Lignin Nanoparticles (I-LNPs)	36
Figure 4.6: Mean Particle Sizes of I-LNPs With Different Volumes of Water (n = 3)	37
Figure 4.7: SEM Micrographs of I-LNPs After Final Addition of (a) 10 mL, (b) 20 mL, (c) 30 mL, (d) 40 mL and (e) 50 mL of Water	38
Figure 4.8: TEM Micrographs of I-LNPs After Final Addition of (a) 10 mL, (b) 20 mL, (c) 30 mL, (d) 40 mL, (e) 50 mL of Water and (f) Particle Sizes Distribution of I-LNPs	39
Figure 4.9: The Morphology of (a) Lignin Nanoparticles and (b) its EDX Spectrum, (c) Iodo-Lignin Nanoparticles and (d) its EDX Spectrum	40

Figure 4.10: SEM Micrographs of (a) LNPs and (b) I-LNPs at 10:1 (v/v) in Water: Acetone After 90 Days of Dispersion	41
Figure 4.11: Stability of (a) LNPs and (b) I-LNPs by Measuring Mean Particle Sizes in Different Volumes of Water:Acetone as a Function of Time	42
Figure 4.12: Mycelial Growth Inhibition of Various Plant Pathogenic Fungi After Treated With 20 nm and 12 mM LNPs	43
Figure 4.13: Mycelial Growth Inhibition of Various Plant Pathogenic Fungi After Treated With 40 nm and 12 mM I-LNPs	47
Figure 4.14: SEM Micrographs of (a) LNPs Treated Bamboo (b) After Leaching, (c) I-LNPs Treated Bamboo (d) After Leaching and (e) Untreated Bamboo	52
Figure 4.15: SEM Micrographs of (a) LNPs Treated Bamboo and (b) its EDX Spectrum, (c) I-LNPs Treated Bamboo and (d) its EDX Spectrum	53
Figure 4.16: Macroscopic Images of (a) B1, (c) W1, and (e) O1 and Microscopic Images of (b) B1, (d) W1 and (f) O1 at 400x Magnification Under Compound Light Microscope	55
Figure 4.17: Agarose Gel Electrophoresis of (a) B1, (b) W1 and (c) O1 PCR Product of Approximately 650 bp. Lane (d) 1Kb DNA Ladder (Transgen)	56
Figure 4.18: Biocidal Efficacy of Unleached and Leached Treated Bamboo Against (a) <i>P. sumatrense</i> , (b) <i>Cunninghamella</i> sp. and (c) <i>Pleosporales</i> sp.	57
Figure 4.19: Mould Growth on Bamboo Inspected on the (a) Front View and (b) Back View	58

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ASTM	American Society for Testing and Materials Standard
AWPA	American Wood Protection Association Standard
CCA	Copper Chrome Arsenic
EDX	Electron Dispersive X-Ray
FT-IR	Fourier Transformed Infra Ray
I-LNPs	Iodo-Lignin Nanoparticles
KI	Potassium Iodide
LNPs	Lignin Nanoparticles
MG	Mean Growth
mM	Millimolar
nm	Nanometre
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscopy
UV	Ultraviolet
UV-vis	Ultraviolet Visible Spectrophotometer

CHAPTER 1

INTRODUCTION

1.1 Study Background

Lignin is the most abundant aromatic polymer and the second most abundant biopolymer after cellulose. It is a complex biopolymer composed of different amounts of monolignols, namely p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Ralph et al., 2019; Schneider et al., 2021). Lignin can be used in industry because it is low-cost, non-toxic, biodegradable, and biocompatible.

Recently, researchers have gained interest on lignin-based nanoparticles due to its improved antifungal activity (Gordobil et al., 2018; Morales et al., 2022). Various functional groups of thiols, phenols and hydroxyl groups make lignin an ideal precursor for functionalisation with various compounds (Cui et al., 2021). Therefore, lignin-based nanoparticles have been applied in various applications such as antioxidant agents (Trevisan & Rezende, 2020), drug delivery carriers (Yiamsawas et al., 2017) and antibacterial agents (Morena et al., 2022). Also, lignin has been reported to exhibit antifungal activity against plant pathogenic fungi. For instance, Gordobil et al. (2018) reported that alkali lignin has weak antifungal activity against pathogenic fungi such as *Aspergillus niger* and *Aspergillus flavus*. Therefore, lignin in combination with another antifungal agent could be used as a promising strategy to enhance its antifungal properties (Jose et al., 2020; Tran et al., 2023).

Meanwhile, Cuellar-Rufino et al. (2022) reported that inorganic compound such as iodine is an effective antiseptic and disinfectant against fungi, viruses, and bacteria. It was reported that iodine and iodocompounds exhibit good antifungal activity at millimolar concentrations against plant pathogenic fungi such as *Fusarium*, *Cunninghamella*,

Aspergillus, and *Penicillium* species (Wei et al., 2022; Wójcik, 2023). However, iodine has several limitations, which include skin discoloration, instability, and irritation. Utilising iodine-binding and releasing polymers (iodophores) can overcome these limitations. For instance, polymer-based iodine such as polyvinylpyrrolidone is a promising way to overcome the limitation of iodine as antiseptic (Pérez-Pazos et al., 2023; Dattilo et al., 2023). Therefore, a combination of lignin nanoparticles and iodine is a great strategy to enhance the antifungal activity of lignin.

The functionalisation of iodine with lignin can be done by using laccase mediated reaction. Laccase is a multicopper oxidase, which is responsible for the oxidation of a wide variety of aromatic and non-aromatic substances (Zerva et al., 2019; Nikolaivits et al., 2021). In the case of lignin, laccase can cleave bond; the bonds between C₁ and C₂ carbons, known as C_α–C_β cleavage, and between C_α and the aryl group, which is known as alkyl–aryl cleavage (Kawai et al., 1999; Zheng et al., 2019). Subsequently, the laccase mediated reaction will bind the aromatic ring of lignin with other inorganic compound such as iodine. The electrons that are produced by the oxidation of the substrate are transferred to molecular oxygen, which is then reduced to potassium hydroxide (KOH) (Sdahl et al., 2019). It is suggested that the utilisation of redox mediator such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) can significantly increases the rates at which iodide oxidation occurs (Li et al., 2019).

The potential of laccase mediated system has further been explored in impregnation of phenolic compound on bamboo. According to Prosper et al. (2016), laccase mediated system could bind iodine on bamboo surface to impart its antifungal resistance. Meanwhile, Wang et al. (2020) demonstrated that laccase was applied to incorporate thymol on the

bamboo surface with high leaching resistance. Therefore, a combination of iodine and lignin nanoparticles could work perfectly well against fungi.

1.2 Problems Statement

Nowadays, plant diseases such as crown rot, leaf blast and scab are serious diseases caused by pathogenic fungi (Jaiswal et al., 2020; Sethy et al., 2020; Baboo et al., 2021; Mondal et al., 2022). Controlling fungal infections via chemical fungicides are not only costly but also can cause environmental problems. For instance, chemical fungicides such as captan, dithiocarbamates and pentachlorophenol are toxic to human health (Vogel et al., 2019; Ranjan et al., 2021; Ajiboye et al., 2022). In addition, the leaching of these chemicals into soils can harm the environment (Yuan et al., 2019; Fanjul-Bolado et al., 2020).

Recently, researchers have gained interest in the application of biopolymer-based nanoparticles derived from lignin as potential antifungal agent. However, lignin is a weak antifungal agent (Gordobil et al., 2018; Jose et al., 2020). Therefore, a combination of lignin with another antifungal agent can enhance its antifungal properties. According to U.S Food and Drug Administration (FDA), iodine can be safely consumed at microgram concentration (Pehrsson et al., 2022). However, iodine is not effective against fungi at microgram/mL or micromolar concentration (Pérez-Pazos et al., 2023). Also, high concentration of iodine as a fungicide is toxic to human and environment. Therefore, functionalisation of lignin-based nanoparticles is essential to improve its antifungal activity.

Apart from that, culm blight, culm rot and witches' broom are serious bamboo diseases caused by rot-fungi (Jiang et al., 2018; Ravi et al., 2022; Lin et al., 2023). Generally, chemical preservatives are costly and toxic to human and environment. For instance, inorganic compounds such as boron can cause serious problems to human health and

environment (Gauss et al., 2020). Besides that, chemical preservatives such as copper chrome arsenic (CCA) and chlorothalonil have carcinogenic properties which can cause serious problems to human health (Kilpi-Koski et al., 2020; Morais et al., 2021). To address these issues, lignin-based nanoparticles are a wise option to be applied as antifungal agents due to its low cost, non-toxic, biodegradable, and biocompatible features.

1.3 Objectives

The specific objectives of this study are:

- i. To synthesise lignin nanoparticles (LNPs) and iodo-lignin nanoparticles (I-LNPs) through nanoprecipitation method in aqueous medium.
- ii. To characterise the surface morphology, chemical and antifungal properties of lignin nanoparticles and iodo-lignin nanoparticles.
- iii. To evaluate the potential application of lignin-based nanoparticles for bamboo protection.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Lignin

Lignin is a complex and irregular biopolymer found in plant secondary cell walls. This aromatic compound consists of monolignol units, the lignin building blocks such as p-hydroxyphenyl, guaiacyl, and syringyl (Schubert et al., 2021; Afifi et al., 2022). According to Weng et al. (2021), these monolignol units are linked via various types of linkages, primarily ether bonds (such as aryl) and carbon-carbon bonds (Figure 2.1). Lignin can be found in vascular plants' middle lamella and secondary cell walls. The cellulose fibrils are encased in lignin and hemicellulose, which give the plant mechanical support and facilitate water conduction (Vanholme et al., 2019). In addition to its structural role, the polyphenolic composition of lignin and its hydrophobic nature ensure the resistance of plants to biological and chemical degradation and contribute to their defence against pathogens (Melro et al., 2021; Shu et al., 2021).

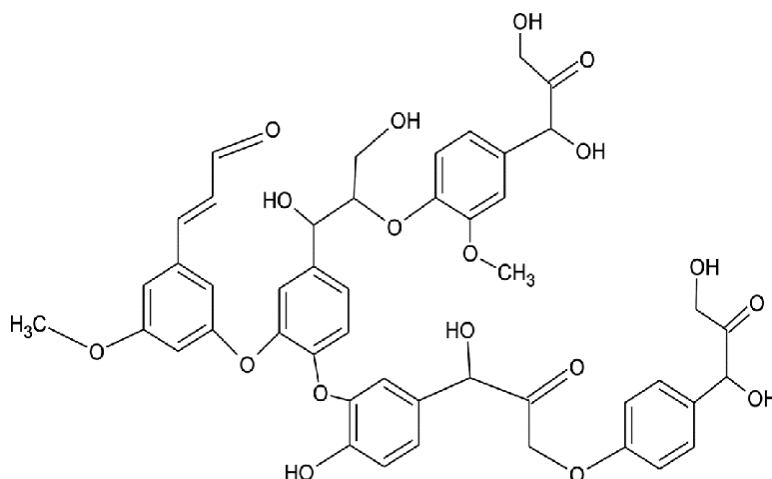


Figure 2.1: Structure of Lignin

The main extraction process of alkali lignin in industry is kraft process (Demuner et al., 2019; Zhao et al., 2019). Kraft process commonly occurs in alkaline media, where lignin is broken into low molecular weight fragments, resulting changes in its physical-chemical properties (Jiang et al., 2019; Acciardo et al., 2022). In kraft procedure, sodium hydroxide and sodium sulphide are added to break the lignin-carbohydrate linkage and give rise to chemically resistant isolated cellulose. This process occurs at a temperature of 180 °C for 2 hours until dark liquor produced, comprising a mixture of inorganic pulping chemicals, hemicellulose, and lignin (Crestini et al., 2017; Budnyak et al., 2020).

The production of alkali lignin by kraft process has a high purity because it contains less derivatives such as sulphur, carbohydrate residues and inorganic impurities (Zhao et al., 2020; Lotfy & Basta, 2022). It also has high antifungal activity compared to organosolv and soda lignin (Morales et al., 2022; Ullah et al., 2022). Due to these properties, alkali lignin is a good choice as raw materials for antifungal study.

2.2 Synthesis of Lignin Nanoparticles

There are several approaches reported in the synthesis of lignin nanoparticles. Several approaches that are widely studied are mechanical and nanoprecipitation methods (Manisekaran et al., 2022; Matsakas et al., 2020; Richter et al., 2016).

2.2.1 Mechanical Method

The high-pressure homogenisation and stirring is a mechanical method usually used in many technical applications due to high efficiency and ease of scaling up. This method could promote the conversion of microsize lignin into nanosize lignin. For instance,

Matsakas et al. (2020) reported that the synthesis of lignin nanoparticles by homogenisation could produce lignin nanoparticles with sizes of 10 nm to 100 nm after 4 hours of mechanical shearing. Similarly, Chang et al. (2019) reported a decreased of nanosize from 200 nm to 100 nm by increasing the stirring rate. In addition, a combination of homogenisation and stirring rate could effectively reduce the particle size. For instance, Mo et al. (2021) reported a decreased in particle size from 300 nm to 100 nm after homogenised and subsequently stirred for 1 hour. Similarly, Tian et al. (2021) demonstrate a decreased of particle size from 1 μ m to 100 nm after homogenisation and stirring at 150 rpm for 1 hour.

In addition, the chemical composition and molecular weight distribution of lignin nanoparticles were not affected after homogenised and stirred (Nair et al., 2014; Wijaya et al., 2021). To conclude, a combination of stirring and homogenisation could effectively decrease the size of lignin nanoparticles.

2.2.2 Nanoprecipitation by Solvent Shifting Method

Solvent shifting is regarded as one of the most promising nanoparticle synthesis methods for leveraging green chemistry in industrial applications due to its low cost and simplicity. Typically, the solvent shifting system to synthesise lignin nanoparticles consists of a mixture of organic solvent and water (Cailotto et al., 2020; Österberg et al., 2020; Ma et al., 2023).

In solvent shifting, lignin was first dissolved into water-miscible solvents such as acetone. The resulting lignin solution was shifted with an excess amount of anti-solvent such as water, leading to the formation of lignin nanoparticles via self-assembly (Richter et al., 2016; Leskinen et al., 2017). According to Ago et al. (2017), the supramolecular interactions

between aromatic lignin structures and internal H-bonding led to formation of spherical lignin nanoparticles via self-assembly (Figure 2.1).

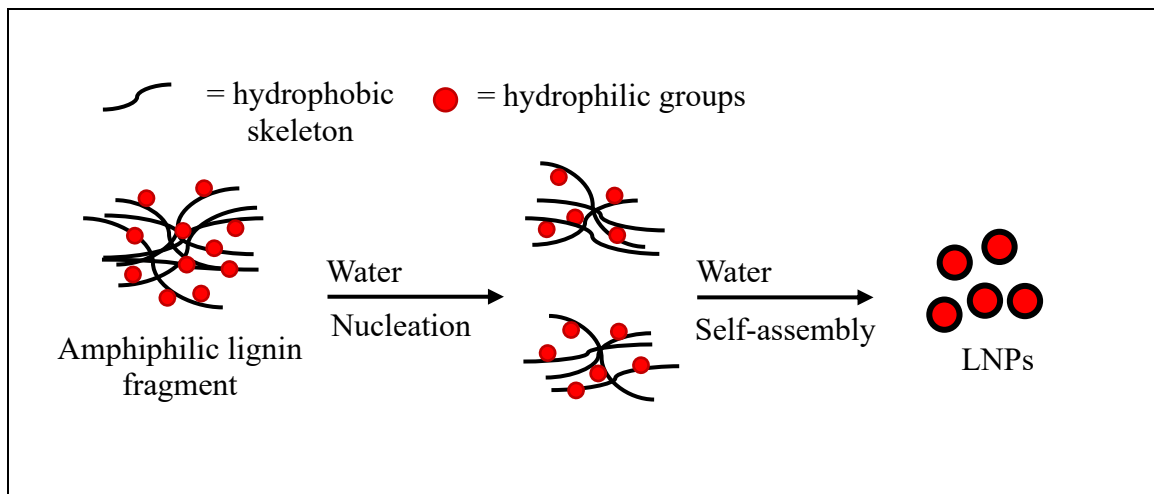


Figure 2.2: The Formation of Lignin Nanoparticles via Self-Assembly (Ago et al., 2017)

In solvent shifting, the size and stability of nanoparticles are affected by the volume of antisolvent, such as water. For instance, Manisekaran et al. (2022) reported a decreased in particles sizes of lignin-based nanoparticles when increased water-to-solvent ratio. The prepared nanoparticles were decreased from 200 nm to 50 nm with increased volume of water from 4 mL to 20 mL. Also, the prepared LNPs by solvent shifting demonstrates superior colloidal stability in water dispersion. Similarly, Lee et al. (2021) prepared spherical LNPs ranged from 100 nm to 20 nm with increasing volume of water in solvent THF. Meanwhile, Richter et al. (2016) prepared LNPs in acetone-water system. They concluded that the prepared LNPs demonstrate a decreased in particle sizes from 300 nm to 50 nm after increasing volume of water from 10 mL to 50 mL of ultrapure water.

In addition, a combination method of solvent shifting, and mechanical stirring could be used to effectively decrease the size of lignin-based nanoparticles. For instance, Lievonen

et al. (2016) prepared LNPs by solvent shifting under slow stirring for 24 h. The prepared LNPs demonstrate decreased in particles sizes from 500 nm to 20 nm. Similarly, Behboudi et al. (2021) reported a decreased in particle size from 200 nm to 10 nm by increasing volume of water and stirring rate. Also, Luo et al. (2021) demonstrated a decrease in particles sizes from 300 nm to 20 nm after homogenised and increasing the stirring rate. The prepared lignin-based nanoparticles also demonstrate excellent colloidal stability when no specific aggregation was observed in water dispersions after 60 days of incubations.

According to Akpinar et al. (2021), small particles sizes on the scale of nanometre could enhance the antifungal activity. For instance, smaller nanosized at 3 nm shows enhanced antifungal activity than 10 nm. Also, nanosized particles demonstrate enhanced antifungal activity than micro-sized particles. For instance, Ferreira et al. (2022) prepared different particle size in the range of 200 nm to 1 μ m. The prepared nanosized particles exhibit greater antifungal activity against *Candida albicans*. Therefore, smaller particle sizes in the scale of nanometre could be used to enhance the antifungal activity of lignin-based nanoparticles.

To conclude, solvent shifting with appropriate volume of solvent and antisolvent can be used to control nanoparticle sizes. Also, a combination of solvent shifting and mechanical method can effectively reduce the particles sizes.

2.3 Functionalisation of Lignin Nanoparticles

The functionalisation of biopolymer via laccase catalysis have gained interest among researchers. Nowadays, there is a continuing need for the potential antifungal agent that is effective, which at the same time possessed low risk to human and environment. The potential of iodine-based compound or known as iodophor has been widely investigated due to wide range of antimicrobial and antifungal activity of elemental iodine (Nakhaei et al., 2021; Makhayeva et al., 2022). One of the most common and widely used antifungal agent is iodo-proparyl butyl carbamate (O'Brien et al., 2019; Raimond et al., 2022).

The functionalisation of lignin nanoparticles could be done via laccase catalysis. Laccase is enzymes that have copper atoms which is responsible for its catalytic mechanisms for lignin degradation and depolymerization (Hilgers et al., 2018; Zheng et al., 2019). Laccase uses oxygen as electron acceptor for oxidation of aromatic and non-aromatic compounds, and generates water as byproducts (Rich et al., 2016). In the case of model phenolic compounds of lignin, laccase cleaves the bonds between C₁ and C₂ carbons, known as C_α-C_β cleavage, and between C_α and the aryl group, which is known as alkyl-aryl cleavage (Kawai et al., 1999; Zheng et al., 2019).

Interestingly, the enzyme laccase is capable of directly oxidising iodide (I⁻) to iodine (I₂) in aqueous solution, which in turn is in equilibrium with triiodide (I₃) (Prosper et al., 2018). According to Sdahl et al. (2019), the molecular iodine formed from potassium iodide (KI) could react with the phenolic lignin to produce iodinated lignin as well as potassium hydroxide (KOH). In addition, laccase-catalysed oxidation of iodide could be enhanced by the addition of redox mediator such as 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) or methyl syringate (Li et al., 2019). The proposed mechanisms of iodo-lignin by electrophilic substitution of aromatic ring is shown in Figure 2.3 (Sdahl et al., 2019).

Another study by Ando and Ralph (2019), also reported that iodination of guaiacyl endgroups in lignin occur at the 5-position of the phenolic ring.

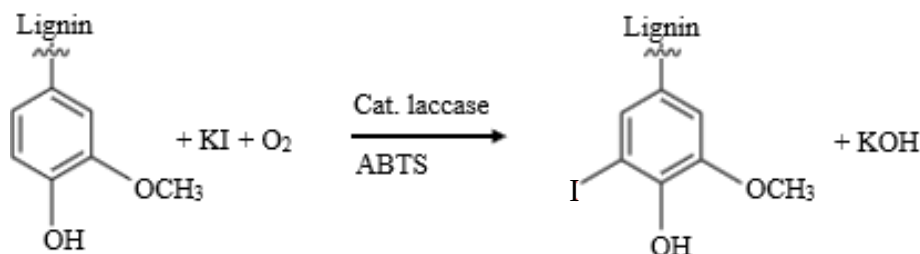


Figure 2.3: Laccase Catalysis of Iodo-Lignin (Sdahl et al., 2019)

2.4 Iodine-based Compound as Antifungal Agent

Iodine is essential component for thyroid hormones which is critical for liver, kidney, muscle and brain functions (Pehrsson et al., 2022). According to U.S. Food and Drugs Administration (FDA), the content of iodine around 20-40 mg iodine/kg of salts is considered as safe to consume (Hatch-McChesney & Lieberman, 2022).

Meanwhile, the recommended concentration of iodine used for disinfectant in plants crops is 4 g/L or approximately 30 mM iodine based on U.S. Environmental Protection Agency (EPA) guidelines (Pérez-Pazos et al., 2023). This is in agreement with Cuellar-Rufino et al. (2022), which reported that Lugol's iodine solutions show minimum inhibitory concentrations at 23 mM against *Candida albicans* and *Candida glabrata*. Similarly, iodine exhibits good antifungal activity against *Acremonium* and *Fusarium* species using 4% iodine or 0.3 M iodine (Vasylchenko & Derevianko, 2021).

In comparison to iodine-based compound, the content of iodine is much lower than aqueous iodine itself. One of the most used iodine-containing compounds is iodophor, complexes of iodine solubilising agent which sustain slow release of free iodine (Gmur &

Karpiński, 2020; Singh et al., 2021; Caracciolo et al., 2023). The enhanced antifungal activity of iodine-based compound is due to synergy effect of iodine and phenolic compounds (Sidahmed & Nimir, 2020). For instance, iodinated phenolic compound, such as iodo-vanillin, has demonstrated superior antifungal activity against *T. versicolor* at millimolar concentrations than with iodine alone (Ihssen et al., 2014). Similarly, polymer-based iodine such as polyvinyl pyrrolidone iodine exhibits good antifungal activity at 12 mM concentrations against *Fusarium*, *Aspergillus*, *Sacroladium* and *Clasdosporium* species on sweet potato plant (*Ipomoea batatas*) (Pérez-Pazos et al., 2023). The biocidal mechanisms of iodine are due to the release of membrane-associated toxicity effect as shown in Figure 2.4 (Navarro et al., 2021).

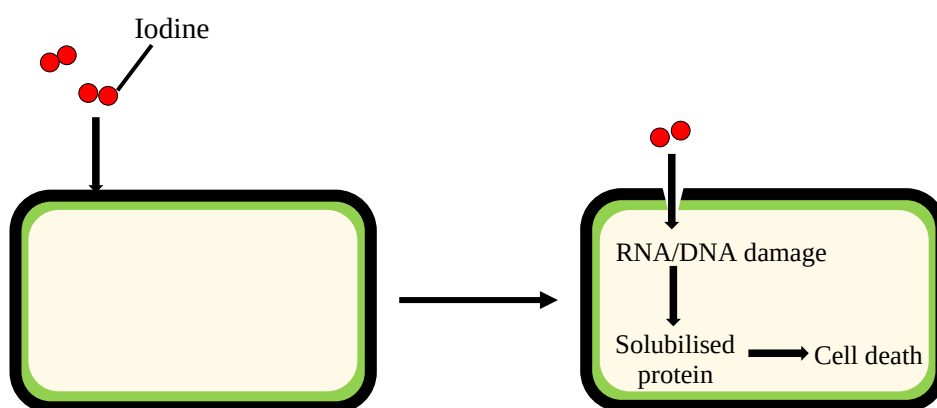


Figure 2.4: Biocidal Mechanisms of Iodine on Cell Wall of Fungi (Navarro et al., 2021)

According to Abueva et al. (2021), iodine could penetrate the cellular membrane of fungi and rapidly initiate its attack on various amino acid groups, particularly cysteine and methionine. Additionally, iodine demonstrates reactivity towards nucleotides and fatty acid groups (Kolasiński, 2019). These rapid biocidal actions would then result in cell rupture and finally cell death (McDonnell & Russell, 1999). To conclude, iodine-based compounds

exhibit excellent antifungal activity against various plant pathogenic fungi. Therefore, the synthesis of iodo-lignin nanoparticles is a promising strategy to enhance the antifungal properties of lignin.

2.5 Plant Pathogenic Fungi

Several pathogenic fungi infecting plant crops are *Fusarium*, *Aspergillus*, *Penicillium*, *Cunninghamella* and *Trichoderma* species. These fungi species can infect food products and cause serious disease to human. *Fusarium* species such as *Fusarium equiseti* has been found to cause wilt in cereal crops such as maize, barley wheat, oats and rice (Lu et al., 2021; Manghwar et al., 2021). Furthermore, it has been found that *F. equiseti* causes wilting disease in chilli and pepper plants (Hami et al., 2021; El-Nagar et al., 2022). According to Perincherry et al. (2019), this species was capable of producing mycotoxins, which has been found to cause skin irritations, diarrhoea, and vomiting upon direct contact with skin, ingestion, or inhalation.

Meanwhile, *Aspergillus flavus* is best known for its colonisations for cereal grains, legumes tree and nuts during storage (Segura-Palacios et al., 2021). In addition, this species can release aflatoxins on the infected fruits which causes serious human disease such as aspergillosis (Rudramurthy et al., 2019; Burks et al., 2021). Meanwhile, *Aspergillus* species such as *Aspergillus niger* has been found to contaminate fruits and vegetables during storage. This species has been reported to cause black mould infections on certain fruits and vegetables such as onions (*Allium cepa*), grapes (*Vitis vinifera*), apples (*Malus domestica*) and peanuts (*Arachis hypogaea*) (Sebaa et al., 2019; Alegbeleye et al., 2022; Kar & Sutar, 2022).

Besides that, *Penicillium* species has been found to cause of decomposition and deterioration of fruits and vegetables. The injuries associated with post-harvest diseases were directly related to mechanical damage, cuts, and wounds during harvest, packing, transport, and storage (Luciano-Rosario et al., 2020). *Penicillium* species such as *Penicillium chermesinum* can cause dark-blue rounded area on corns (*Zea mays*) and grapes (*Vitis vinifera*) (El-Samawaty et al., 2021; Sun et al., 2021; Laut et al., 2023). Additionally, this species produces harmful mycotoxins and carcinogenic compounds which cause serious problems to human health (Patriarca, 2019; Otero et al., 2020).

Cunninghamella species is commonly found in a variety of substances such as vegetables fruits, nuts, crops, and animal wastes. Mucormycosis is a fungal disease caused by *Cunninghamella* species (Hallur et al., 2021; Mita et al., 2022). The symptoms of this disease may include headache, fever and crusting of nasal mucosa (Serris et al., 2019; Soliman et al., 2021). On the other hand, *Trichoderma* species such as *Trichoderma afroharzianum* has been found to cause degradation of plant tissue on maize plants (Alvindia, 2018). Also, this species has been reported to cause allergic reaction to immunocompromised individuals (Lu et al., 2016).

To conclude, plant pathogenic fungi can cause severe damage to plant crops especially during harvest, storage or transport. Therefore, the evaluation of lignin-based nanoparticles against various species of fungi are essential to determine its effectiveness as a potential nanobiocide.

2.6 Background of Bamboo

In regions including Southeast Asia, India, South America, and Central America, bamboo is a significant primary resource for the construction, furniture, and paper industries (Lee et al., 2023). The local population residing in this region has relied heavily on bamboo for their economic and subsistence needs. This material has a variety of commercial applications, including but not limited to construction materials, paper pulping, fencing, cooking utensils, water pipelines, low-rise housing, and bridges (Chen et al., 2022; Fahim et al., 2022).

One of the most important features of bamboo is that it can rapidly reach maturity in 3 years, while wood take at least 20 years to reach maturity (Guleria et al., 2020). Despite having some disadvantages, such as difficult for modelling due to hard tissues and hard texture, it still has important characteristics such as high tensile strength and water resistance materials (Emamverdian, 2020; Hassan et al., 2020; Dessalegn et al., 2022). However, unlike wood, bamboo has high content of sugar and starch which can easily be attacked by mould fungi (Dai et al., 2017; Mohan et al., 2022). Therefore, bamboo needs to be treated to prevent mould infections.

2.6.1 *Dendrocalamus asper*

Dendrocalamus asper or locally known as *buluh betong* is a multipurpose, tropical, clustering bamboo with a high economic value (Kong et al., 2020; Mustafa et al., 2021). It is also referred to as rough bamboo or gigantic bamboo (Karlinasari et al., 2021). As shown in Figure 2.5, *Dendrocalamus* species can attain a height of 20-30 m, has a diameter of 8-20 cm and 20-45 cm long-internodes, and has relatively thick walls (Hossain et al., 2018). According to Ahmad et al. (2021), *D. asper* could be found throughout India and Southeast Asia, including Thailand, Vietnam, Malaysia, Indonesia, and Philippines.



Figure 2.5: The Image of *Dendrocalamus asper*

Although the origins of *D. asper* remain unclear, it is known that they could be found in these regions (Mustafa et al., 2021). Within tropical Asia, the best environment for the growth of *D. asper* is a humid climate with rich, heavy soils, ranging from lowlands to an

altitude of 1500 metres, and receiving an annual rainfall of approximately 2400 millimetres (Mustafa et al., 2021; Masisi et al., 2022). It is also able to thrive in areas that are semiarid when given the appropriate care (Govindaraj & Robinson, 2020).

Dendrocalamus species especially *D. asper* serves significance economic value to local people. According to Hartono et al. (2022), its upper internodes are used to manufacture containers and cooking pots. Meanwhile, the mature stems are used to create paper, furniture, musical instruments, domestic items, handicrafts (Singh et al., 2020; Chaowana et al., 2021; Kumar et al., 2023). As a vegetable, the young shoots are eaten when they are still tender, and they are considered as the finest in taste of all the tropical Asian bamboo (Felisberto et al., 2021).

Nevertheless, *D. asper* has high contents of sugar and starch which can be easily attacked by fungi in moist environment (Silva et al., 2020; Subekti & Yoshimura, 2020). For instance, *D. asper* can easily decay and discoloured by mould fungi in moist environment (Subekti & Yoshimura, 2020). Therefore, further pre-treatment after harvesting is needed to extend its life service.

2.7 Bamboo Treatment Method

There are several approaches to preserve bamboo including nanoparticle impregnation, harvesting practise and chemical preservation (Ginoble Pandoli et al., 2019; Liao et al., 2021; Tafa & Engida, 2022).

2.7.1 Harvesting Practises

Bamboo's susceptibility to fungi can be influenced by the time of harvesting. According to Subekti and Yoshimura (2020), bamboo contains large amount of starch which

could be principal nutrient for parasites and fungi. Thus, the best time to harvest bamboo is after rainy season. For instance, *Dendrocalamus asper* is best to harvest during dry season because of lower sugar content (Dessaiegn et al., 2022). It is also recommended to harvest mature stems of *D. asper* that are 3 to 5 years old due to lower starch content (Darabant et al., 2016). For storage, the culms of bamboo should be cut, while the leaves are not removed to allow natural evaporation of free capillary of water (Chandel et al., 2018; Chaowana et al., 2021).

2.7.2 Nanoparticles Impregnation

Nowadays, researchers have gained interest on the application of nanotechnology in wood and bamboo, while the application in bamboo is not adequate so far (Papadopoulos & Taghiyari, 2019). The nanoparticles can be impregnated into bamboo by several methods such as hydrothermal, sol-gel method and laccase catalysis.

Hydrothermal method is a high-temperature heat treatment to modify the surface of wood and bamboo (Ali et al., 2021; Yuan et al., 2021). According to Ringman et al. (2014), the improved decay resistance of modified wood and bamboo was mainly due to less nutrient available and reduced moisture content after high-temperature heat treatment. This physical method is usually carried out in a high-pressure batch reactor at temperatures between 160-190 °C to reduce the content of cellulose and hemicellulose on bamboo (Ma et al 2021; Lv et al., 2022). For instance, Yuan et al. (2022) treated bamboo at temperature ranging from 120-160 °C to reduce the content of cellulose and hemicellulose on bamboo. They concluded that the treated bamboo exhibit increased antifungal resistance than untreated bamboo. In contrast, Ren et al. (2018) developed a two-step approach for preserving bamboo. For instance, they employed a low heat temperature of 60 °C for 24 hours followed by spraying

antifungal agent on bamboo. They concluded that the prepared bamboo outperforms hydrothermally treated bamboo in terms of antifungal resistance. However, this strategy is costly in terms of energy consumption (Jiang et al., 2023).

Meanwhile, sol-gel method is a chemical method to deposit nanoparticles in the form of gel on bamboo surfaces (Li et al., 2019; Yang et al., 2020). Generally, the bamboo are performed in Soxhlet extraction in a diluted aqueous sodium hydroxide following addition of water to activate cellulose for improves interaction with impregnating agent. Then, nanomaterials such as metal alkoxide are dissolved in water or alcohol to form gel by heating and subsequent stirring (Sun et al., 2019; Qu et al., 2021; Andriulo et al., 2022; Hou et al., 2023). The advantage of this treatment is that the bamboo demonstrates excellent resistance to fire and ultraviolet (UV) degradation. However, this method is not yet feasible for scale up due to high cost of operations (Sun et al., 2019).

In laccase catalysis method, laccase enzyme is employed to modify the bamboo surface (Kenned et al., 2021; Bi et al., 2022; Chen et al., 2023). This surface modification of bamboo fibre is also known as grafting method. In grafting method, hydroxyl groups lignin is oxidised by laccase, thus promoting the crosslinking with impregnating agent (Dong et al., 2021; Wu et al., 2022). This enzymatic reaction uses oxygen for substrate oxidation and generate only water as side-product (Li et al., 2022). In addition, the activity of laccase can be enhanced using redox mediator such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The combination of laccase and ABTS is also known as laccase mediated system. For instance, Martin et al. (2022) treated bamboo via laccase mediated systems to improve mechanical properties of wood fibreboard. They concluded that lignin binds to vanillin more effectively via laccase-mediated system than that of without mediator. Similarly, Garajová et al. (2021) prepared lignin-based composite by laccase mediated

systems. They concluded that the laccase mediated systems could be used to reinforce lignin-based composites on bamboo surface.

Also, laccase mediated system could be used to bind inorganic compound on bamboo fibre. For instance, Prosper et al. (2018) treated bamboo via laccase catalysis to bind potassium iodide (KI) on bamboo. It was observed that lignin demonstrates the greatest changes in FT-IR, especially relative to the methoxyl groups and aromatic skeleton after modification. Meanwhile, no structural modifications were observed for cellulose and hemicellulose from bamboo. They concluded that lignin was oxidised by laccase and subsequently promote the formation of I–C bonds on bamboo surface. Moreover, the treated bamboo demonstrates excellent antifungal resistance against *Aspergillus niger*.

To conclude, laccase mediated systems is a promising strategy to deposit nanoparticles on bamboo due to its simplicity and high leaching resistance. Also, it is considered as eco-friendly method because oxygen is used for substrate oxidation and generate only water as side product.

2.7.3 Chemical Preservation

Boric acid is an inorganic acid used to treat bamboo for indoor applications. According to Gauss et al. (2020), bamboo treated with 2% boric acid shows almost complete protection from plant pathogenic fungi. Meanwhile, a combination of boric acid in aluminosilicate films demonstrates enhanced antifungal activity due to synergistic effect of boric acid and the inorganic films (Yves et al., 2020). However, the application of boric acid is limited to indoor application due to higher leaching of boric acid with water (Wu et al., 2022; Duan et al., 2023).

Several chemical preservatives such as copper chrome arsenic (CCA) and IPBC (3-iodo-2-propenyl butyl carbamate) are known for bamboo treatment. These preservatives can effectively treat wood or bamboo against decay fungi. For instance, CCA and IPBC have demonstrated almost complete inhibition against mould and decay fungi (Yang et al., 2019; Han et al., 2022). However, CCA contains arsenic and chromium compounds, which are harmful to human due to their carcinogenicity (Kato et al., 2021; Scussel et al., 2022; Villegas et al., 2023). Meanwhile, treatment with IPBC is highly toxic and not effective against water leaching (Zhang et al., 2020; Hwang et al., 2021). Therefore, the use of chemical preservatives is not recommended because it is highly toxic to human. Further, leaching of these chemicals from soil into groundwater and surface water can cause serious water pollutions (Ranjan et al., 2021).

2.8 Fungi Infecting Bamboo

Bamboo is regarded as important alternative source for wood-based products. However, bamboo can easily be attacked by mould and decay fungi due to high sugar and starch content (Figure 2.6). There are several pathogenic fungi species that have been infecting bamboo such as *Penicillium*, *Cunninghamella* and *Pleosporales* species (Shen et al., 2014; Subekti & Yoshimura, 2020).

According to Yan et al. (2021), *Penicillium* species such as *Penicillium citrinum* has been found to cause severe mould growth and decay to bamboo culms. Similarly, Abreham et al. (2015) reported that this species was the main cause of root rots and decay on Acacia tree. Besides that, *Cunninghamella* species such as *Cunninghamella elegans* has been found to cause root rots in cassava plant (*Manihot esculenta Crantz*) (Pham & Tran, 2021). On the

other hand, *Pleosporales* species has been reported to cause decay on bamboo and palm trees (Phookamsak et al., 2022).



Figure 2.6: The Image of *Dendrocalamus asper* Infected by Plant Pathogenic Fungi

To conclude, the presence of pathogenic fungi can lead to significant disease development on bamboo. Therefore, it is essential to implement effective control measures to preserve its longevity and quality.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Alkali lignin, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potato dextrose agar (PDA), potato dextrose broth (PDB), *T. versicolor* laccase and lactophenol cotton blue were purchased from Sigma-Aldrich. Acetone, chloramphenicol, potassium iodide (KI), McIlvain buffer pH 5 were purchased from Bendosen. Various species of fungi namely, *F. equiseti*, *Cunninghamella* sp., *P. chermesinum*, *A. niger*, *A. flavus*, and *T. piluliferum* were provided by Biomolecular Lab, Universiti Malaysia Sarawak. Bamboo samples 50 x 50 x 20 (radial) mm³ were produced from 4-year-old *Dendrocalamus asper* culms collected from Plant Research Centre, Universiti Malaysia Sarawak. Ultrapure water (~18.2 MW, 25 °C) was obtained from a Water Purifying System (ELGA, Model Ultra Genetic). All chemicals were used as received without further purification.

3.2 Preparation of Lignin Nanoparticles

Lignin nanoparticles (LNPs) were prepared by solvent shifting in acetone-water mixture with slight modification (Richter et al., 2016). 2.0 g of alkali lignin was dissolved in 200 mL of 70% acetone under ambient conditions. Then, 1 mL of alkali lignin solution was added dropwise into 10 mL of ultrapure water to form ratio of water: acetone, 10:1(v/v). Afterwards, the mixture was homogenised for 1 hour. Then, the mixture was continually stirred at 150 rpm for 1 hour. The steps were repeated with lignin in different ratio of water:

acetone such as 20:1, 30:1, 40:1 and 50:1 (v/v) to obtain different sizes of LNPs. Afterwards, the solid samples were collected through centrifugation and oven dried at 60 °C for 2 hours.

3.3 Preparation of Iodine Functionalised Lignin Nanoparticles

Iodination of LNPs was carried out by laccase catalysed iodination with a slight modification method (Ihssen et al., 2014). The laccase catalysed reactions were carried in McIlvain buffer pH 5 containing 20 mM of LNPs, 10 mM (2,2'-azino-bis(3 ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 50 mM potassium iodide (KI).

The preparation of I-LNPs was first done by adding 2.0 g of LNPs to a final concentration of 20 mM in 100 mL buffer. Afterwards, 100 µL of 4 U/mL laccase was added to the LNPs solutions. Then, 100 mL of 50 mM potassium iodide was added to make up of 200 mL solutions in Erlenmeyer flask. Subsequently, 100 µL of ABTS was added to the reaction mixtures using micropipette. The steps were repeated with different sizes of LNPs at 220 nm, 130 nm, 80 nm, 55 nm and 20 nm. Then, the reaction mixtures were continually stirred for 18 hours under ambient conditions. The solid samples were collected through centrifugation and oven dried at 60 °C for 2 hours.

3.4 Characterisation of Samples

Samples were characterised by using Fourier Transformed Infrared spectrophotometer (FT-IR, Thermo Scientific Nicolet iS10) with potassium bromide within the wavenumber range of 4000-500 cm⁻¹. The morphologies of nanoparticles were observed using scanning electron microscopy (FESEM, JSM-IT 500 HR) coupled with energy dispersive X-ray (EDX) spectroscopy. The mean diameter of nanoparticles was determined

using the Smile View software by measuring the diameter of at least 50 spherical nanoparticles as observed in the SEM micrographs. A measured expanse of nanoparticles was dispersed in absolute acetone and a drop of the dispersed sample was drop coated onto formvar-coated copper grids. TEM micrographs of nanoparticles were obtained using a transmission electron microscope (TEM) (JEOL Model1230). The absorbance of nanoparticles was measured using ultraviolet-visible spectrophotometer (UV-vis, 1900I) with ethyl acetate as a blank within the wavenumber range of 500-250 cm⁻¹.

3.5 Antifungal Assay

The antifungal activity of LNPs and I-LNPs was evaluated against fungi such as *Cunninghamella* sp., *F. equiseti*, *P. chermesinum*, *A. flavus*, *A. niger* and *T. piluliferum*. Various nanosizes of LNPs and I-LNPs were added to the potato dextrose agar (PDA) at 3, 6, 9 and 12 mM. The petri dish with agar was inoculated with grown fungus (4 mm diameter) at the centre of the Petri dish and incubated for 14 days under ambient conditions.

The control plate was inoculated with fungi without treatment. All experiments were carried out in triplicates. The percentage of inhibition was calculated and the mean values were analysed using one-way ANOVA (Tukey's Test) with significance at $p < 0.05$ by using IBM SPSS software.

The percentage inhibition of fungi was calculated from Equation 3.1:

$$\text{Growth inhibition(\%)} = \frac{\text{MG in control} - \text{MG in treatment}}{\text{MG in control}} \times 100\% \quad \text{Equation 3.1}$$

*Mean growth (MG) on the day when the control plate is full

3.6 Preparation of Bamboo

LNPs-treated bamboo was prepared via laccase mediated systems (Figure 3.1). The preparation was first done by the addition of 1.0 g lignin in 100 mL of water and 2 mL of acetone and in the ratio of 50:1 (v/v). Then, the solution was stirred at 150 rpm for 1 hour using a homogeniser. Next, the bamboo blocks were immersed in the treatment solution containing 100 mL of buffer with triplicates each. The prepared bamboo blocks were submerged with weights to assure complete immersion in the reaction mixture. Subsequently, 50 μ L of laccase (10 U/mL) and 300 μ L of ABTS (10 mM) were added to the reaction mixtures. The solution was incubated at 40 °C on water bath for 18 hours. Afterwards, the bamboo blocks were removed from solutions and oven-dried at 40 °C for 2 hours.

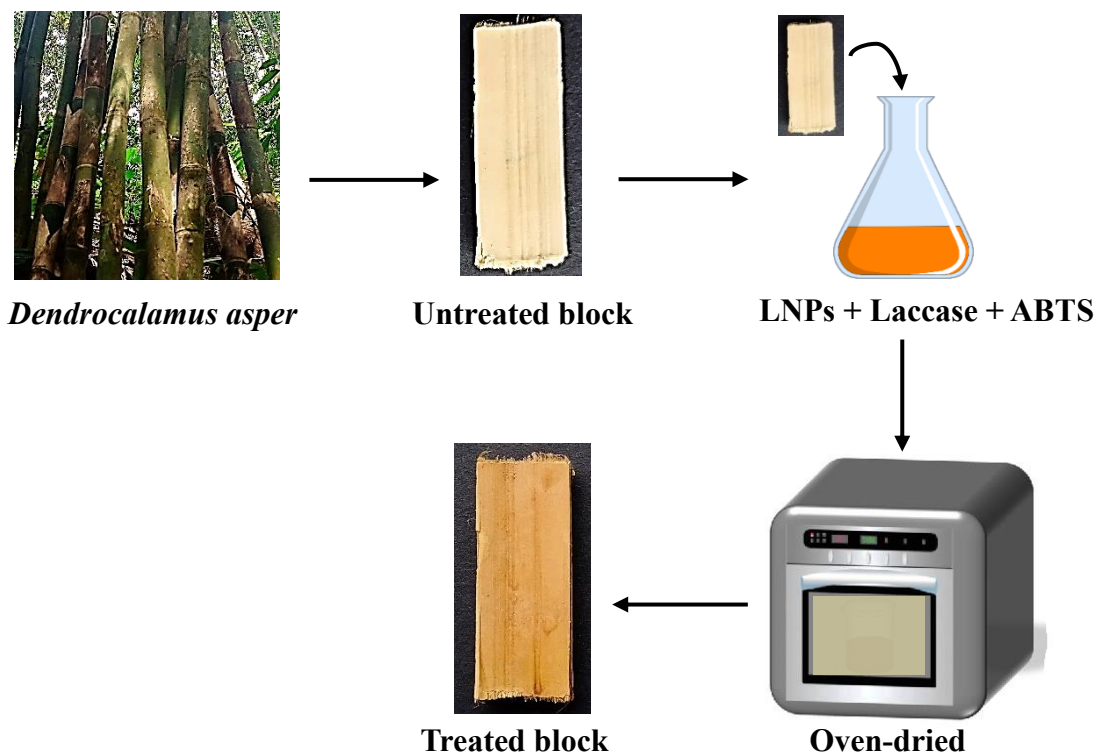


Figure 3.1: Preparation of Bamboo by Laccase-Catalysed Oxidation of LNPs and I-LNPs

I-LNPs-treated bamboo was prepared by laccase catalysed iodination method according to Ihssen et al. (2014) with slight modification. 1.0 g of LNPs was dissolved in 50 mL of water and 1 mL of acetone to form a ratio of 50:1 (v/v). The reaction was made up of 200 mL solution in an Erlenmeyer flask containing 100 mL of potassium iodide (100 mM), 50 mL of McIlvan buffer pH 5, 10 U/mL of *T. versicolor* laccase and 300 μ L of ABTS (10 mM). Then, bamboo blocks were immersed in the treatment solution with triplicates each. Afterwards, the bamboo blocks were removed from solutions and oven-dried at 40 °C for 2 hours. Then, the morphologies of treated and untreated bamboo samples were characterised using SEM and EDX analyses.

3.7 Isolation of Fungi from Bamboo

The fungi were isolated from the bamboo culm of *Dendrocalamus asper*. The isolations were firstly done by swabbing the surface of the infected bamboo using sterile cotton swabs and kept inside separate sterile tubes prior to isolation. In laboratory, the collected samples were inoculated onto plates of potato dextrose agar (PDA) supplemented with chloramphenicol to prevent bacterial growth. The plates were incubated at 28 °C for 5 days. Then, the fungi were subcultured in PDA and incubated for 7 days to obtain a pure culture. All the pure colonies were subcultured again in slant agar of PDA. The cultures were stored at 4 °C until further used (Adeoye & Lateef, 2021).

To prepare spore suspension of fungi, the fungi were cultured in potato dextrose broth (PDB) and incubated at room temperature under shaker for 5 days. Then, the spore suspension was completely homogenized using tube shaker and filtered. The suspensions were then kept in sterile vials at 4 °C for antifungal tests on bamboo.

3.8 Molecular Identification of Fungi

Genomic DNA of the isolated fungus was extracted using the method described by Cubero et al. (1999). Subsequently, the extracted fungal DNA was subjected to PCR amplification with the universal primer pair ITS1 and ITS4, under the following conditions: initial denaturation at 95 °C for 5 minutes, 30 cycles of denaturation at 95 °C for 1 minute, annealing at 55 °C for 1 minute, extension at 72 °C for 1 minute, and a final extension at 72 °C for 7 minutes. The PCR products were then purified and subjected to sequencing. Close homologous sequences of the isolates were obtained from the NCBI GenBank database.

3.9 Mould Test and Characterisation

Mould resistance tests of bamboo were evaluated according to American Society for Testing and Material D4445-10 standard (ASTM D4445-10, 2019). The prepared bamboo block was firstly dipped into spore suspension of fungi. Then, the bamboo block was placed in a petri dish to air dry for 24 hours.

Meanwhile, the leaching test was first performed by submerging the treated blocks in 100 mL of distilled water and replacing the water every 24 hours over a period of 2 weeks. The bamboo blocks were labelled as control, unleached LNPs (LNPs-UL), unleached I-LNPs (ILNPS-UL), leached LNPs (LNPs-L) and leached I-LNPs (ILNPs-L) with triplicates each. The untreated bamboo samples were indicated as control specimens.

All petri dishes with bamboo were incubated using incubator (Mettler, Model 30-1060) at 28 °C and 85% of relative humidity. During incubation, the bamboo surface was visually inspected for the development of mould growth according to 5-point criteria ranking in Table 3.1.

Table 3.1: Ranking System for Specimen Mould Damage (AWPA E-10, 2020)

Value	Infection area of specimen
0	No mycelium on the surface
1	The surface area of fungi infection < 25%
2	The surface area of fungi infection 25-50%
3	The surface area of fungi infection 50-75%
4	The surface area of fungi infection >75%

The mould growth was inspected on weekly basis for 28 days. The lower the infection values the better biocidal efficacy against fungi.

The biocidal efficacy of fungi was calculated from Equation 3.2:

$$\text{Biocidal efficacy} = (1 - D_t / D_o) \times 100\% \quad \text{Equation 3.2}$$

where D_t was an average infection of the test specimens, D_o was an average infection of control specimen.

3.10 Mass Loss of Bamboo

The test samples were dipped into the spore suspension of various pathogenic fungi species and placed in a petri dish. The assay was triplicates each. Next, petri dishes with samples were incubated at 28 °C and a relative humidity of 85%. Then, decay test was determined gravimetrically after incubation periods of 12 weeks and expressed as mass loss (%) according to American Wood Protection Association E10 standard (AWPA E-10, 2020).

The mass loss of bamboo was calculated from Equation 3.3:

$$\text{Mass loss (\%)} = [(m_0 - m_1) / m_0] \times 100\% \quad \text{Equation 3.3}$$

where m_0 is the dry weight of the bamboo specimen before treatment and m_1 is the dry weight of the bamboo specimen after treatment.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterisation of LNPs via SEM and TEM Analyses

The lignin nanoparticles were formed via a nucleation-growth mechanism as the water was added to the acetone during synthesis. In this mechanism, the size of LNPs depends on the supersaturation of lignin concentration in the acetone-water (Manisekaran et al., 2022). In the present study, the solubility of lignin in solution increased as the ratio of water to acetone (v/v) increased, resulting in a solution that was supersaturated. According to Domínguez-Robles et al. (2018), alkali lignin has high solubility in diluted acetone. Therefore, the addition of more water to acetone resulted in homogeneous nucleation, which produced lignin nanoparticles with smaller diameter (Figure 4.1).

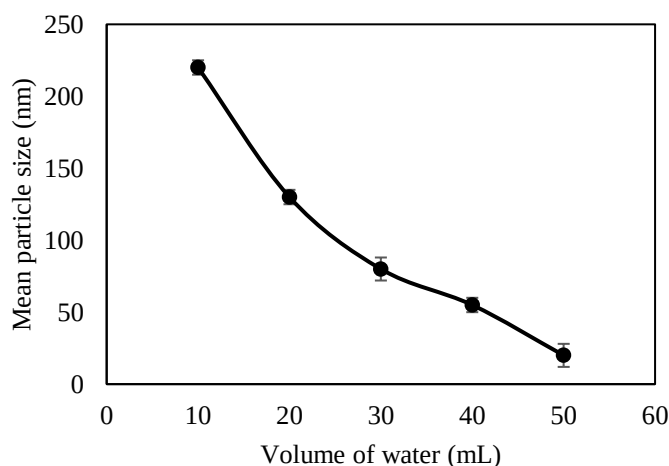


Figure 4.1: Mean Particle Sizes of LNPs With Different Volumes of Water (n = 3)

Figure 4.2 (a-e) shows the SEM micrographs of lignin nanoparticles. As the volume of antisolvent (water) increased from 10 mL to 50 mL (Figure 4.1), it was found that the average particle sizes of LNPs was affected by the volume of antisolvent (water) during the

synthesis as proven by the decrease of mean particle sizes from 220 nm to 20 nm. At lower volume of water, LNPs showed larger particle size distribution, more aggregated, and not uniformly dispersed. However, at higher volume of water, LNPs produced uniform homogeneous dispersion and smaller nanoparticles.

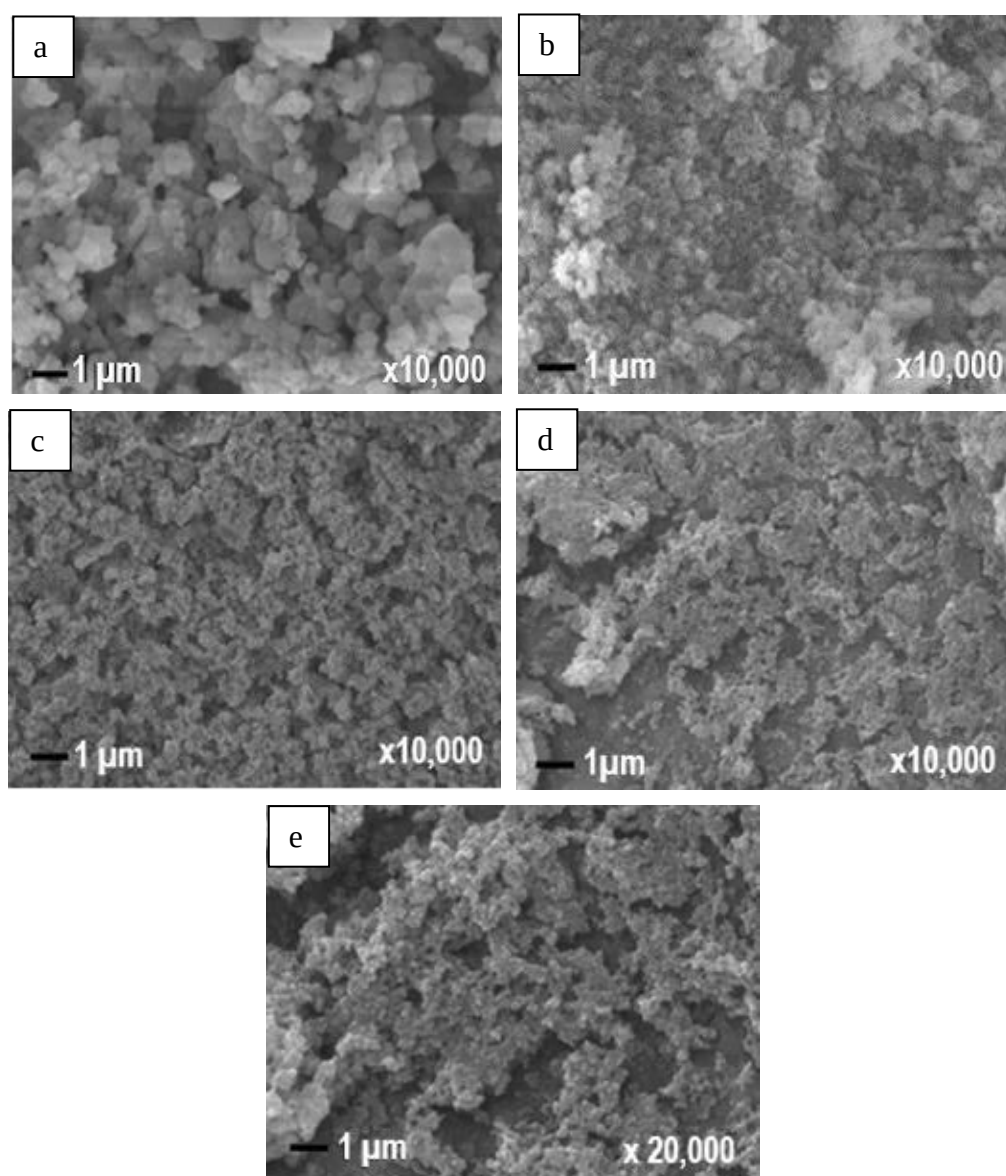


Figure 4.2: SEM Micrographs of LNPs After Final Addition of (a) 10 mL, (b) 20 mL, (c) 30 mL, (d) 40 mL and (e) 50 mL of Water

Based on Figure 4.3, spherical image of LNPs were formed during nanoprecipitation method. According to Akpinar et al. (2021), smaller particles size of copper around 300 nm to 10 nm were reported to be effective against plant pathogenic fungi. This is because copper nanoparticles allowed easy penetration into cell wall of fungi due to higher surface area-to-volume ratio.

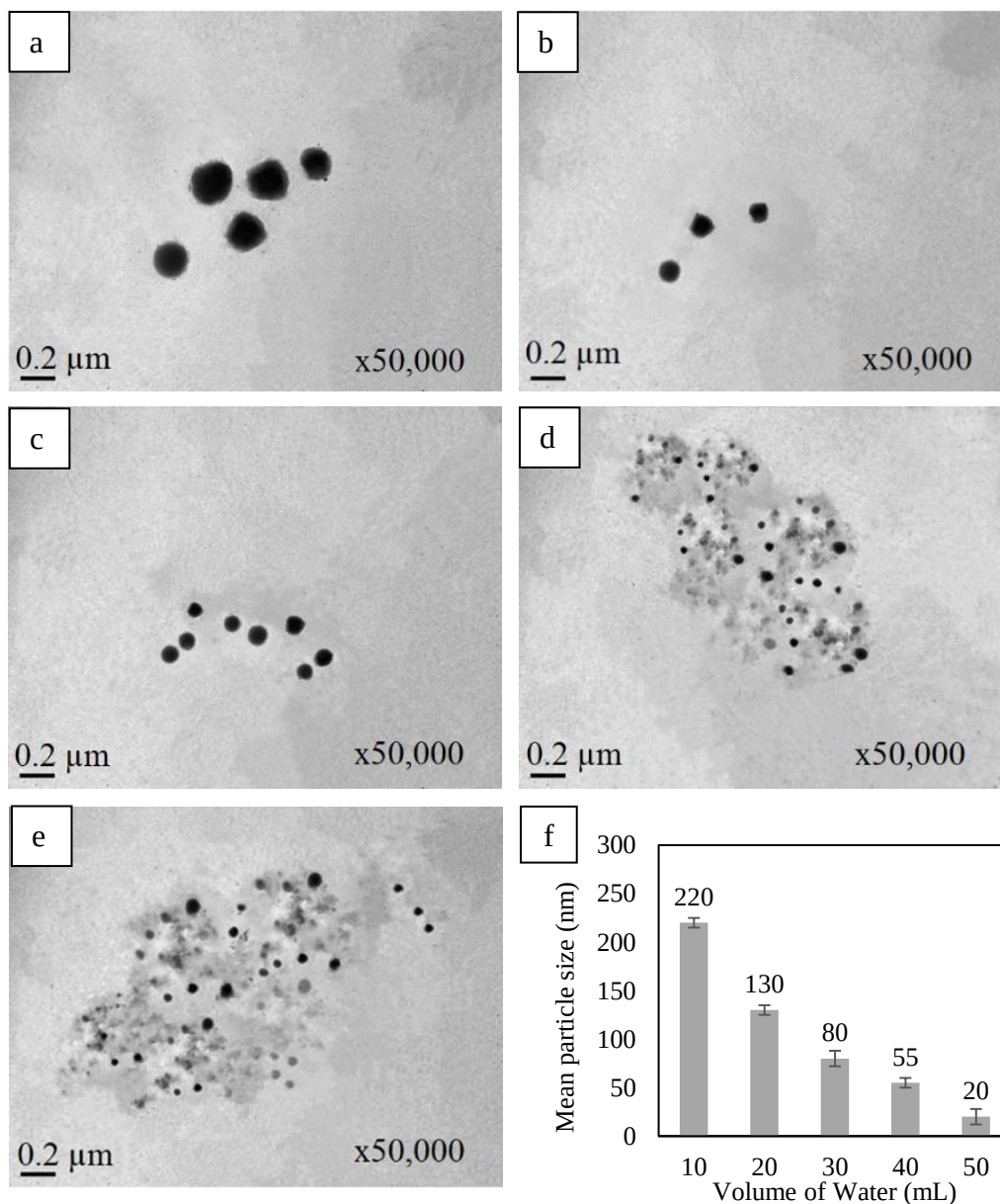


Figure 4.3: TEM Micrographs of LNPs After Final Addition of (a) 10 mL, (b) 20 mL, (c) 30 mL, (d) 40 mL, (e) 50 mL of Water and (f) Particle Sizes Distribution of LNPs

4.2 UV-vis Analysis of I-LNPs

The formation of I-LNPs was further observed under UV-vis analysis after 18 h of incubation. The reaction was carried out in 50 mM of KI, in the presence of 4 U/mL laccase and ABTS. As shown in Figure 4.4, the absorption peak of lignin nanoparticles (LNPs) was located at 280 nm due to B-band (benzenoid band) of π - π^* transitions of the aromatics group in lignin. Comparatively, new absorption peaks of iodo-lignin nanoparticles (I-LNPs) at 288 nm and 366 nm were formed when lignin nanoparticles (LNPs) was subjected to iodination.

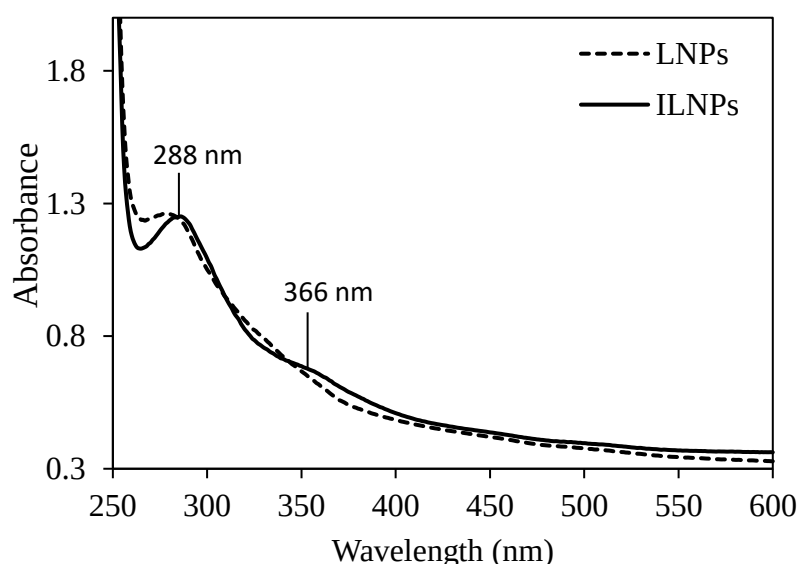


Figure 4.4: UV-vis Spectra of LNPs and I-LNPs

The absorption peak of LNPs at 288 nm results from overlapping of the characteristic absorption of lignin and the charge-transfer transition bands of lignin-iodine complexes (Dai et al., 2018). Therefore, charge-transfer transition peak located at 288 nm was formed because charge-transfer transition occurred from the highest occupied molecular orbital (HOMO) of the aromatic groups of lignin to the lowest unoccupied molecular orbital (LUMO) of iodine (Grozema et al., 1999). Meanwhile, the absorption peak of I-LNPs at 366

nm was attributed to the formation of triiodide (I_3^-) ion in the solution of lignin-iodine complexes (Weng et al., 2006). To conclude, I-LNPs was successfully formed using laccase mediated system consists of KI and ABTS as redox mediator.

4.3 FT-IR Spectroscopy

The functionalisation of lignin-based nanoparticles with iodine was further confirmed by FT-IR spectroscopy analysis. Based on Figure 4.5 (a), the parent lignin exhibits wide absorption band in the 3700 to 3050 cm^{-1} range which is typically associated with stretching of -OH groups. Besides that, the peak at 1732 cm^{-1} and 1695 cm^{-1} were attributed to the stretchings of carbonyl group conjugated with aromatic ring of lignin (Coates, 2006). The parent lignin showed absorption in the wavenumber range of 1600 to 1400 cm^{-1} due to carbon to carbon ($C=C$) stretchings vibration in the aromatic ring (Coates, 2006). Meanwhile, the peak at 2924 cm^{-1} and 2852 cm^{-1} were assigned to the methylene C-H asymmetric and symmetric stretchings of lignin, respectively (Salim et al., 2021). The peak at 850 cm^{-1} is attributed to the π - π conjugated structure of aromatic ring of lignin.

In the present study, the laccase catalysed iodination results in cleavage of carbon-carbon and aromatic rings in lignin (Bourbonnais et al., 1995; Kawai et al., 1999). As shown in Figure 4.8 (b), the peak at 849 cm^{-1} was weakened indicating the π - π conjugated structure of I-substituted ring of lignin was formed. Similarly, Dai et al. (2018) reported that the peak at 849 cm^{-1} was weakened after iodination treatment.

Moreover, the new peak at 1087 cm^{-1} assigned to I-substituted ring was formed (Prosper et al., 2018). In the case of lignin, I-substituted ring can be shifted to higher frequency than the aliphatic C-I bonds (500–600 cm^{-1}) (Coates, 2006). Although no well-

defined I-substituted aromatic compounds are observed, the presence of a halogen on an aromatic ring can be detected indirectly from its electronic impact on the C-H bands (Coates, 2006; Prosper et al., 2018). The bands 2910 cm^{-1} and 2842 cm^{-1} assigned for C–H stretches of side chain CH_3 and CH_2 were increased after iodination of LNPs.

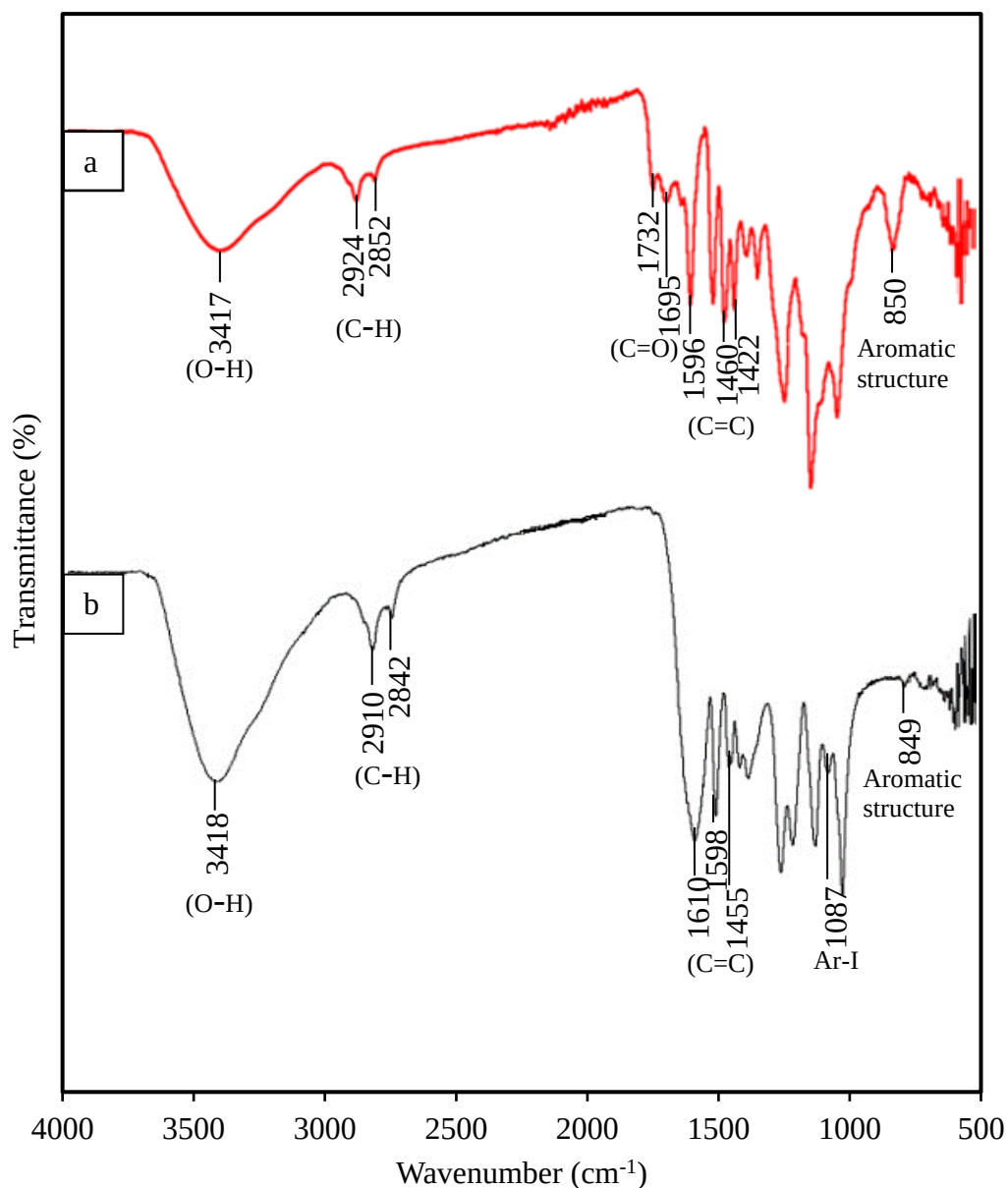


Figure 4.5: FT-IR Spectroscopy of (a) Lignin Nanoparticles (LNPs) and (b) Iodo-Lignin Nanoparticles (I-LNPs)

4.4 Characterisation of I-LNPs via SEM and TEM Analyses

As the volume of antisolvent (water) increased from 10 mL to 50 mL (Figure 4.6), it was found that the average particle size of I-LNPs decreased from 360 nm to 40 nm. Similarly, at lower volume of water, I-LNPs were produced to give more aggregated and larger particle size distribution, and also not dispersed uniformly (Figure 4.7). However, at high volume of water, I-LNPs could give rise to more uniform homogeneous dispersion and smaller nanoparticles. Comparatively, the sizes of I-LNPs were slightly larger than LNPs after being prepared by nanoprecipitation method. This was probably due to the functionalisation of iodine on lignin after laccase-catalysed oxidation (Ihsen et al., 2014; Manisekaran et al., 2022).

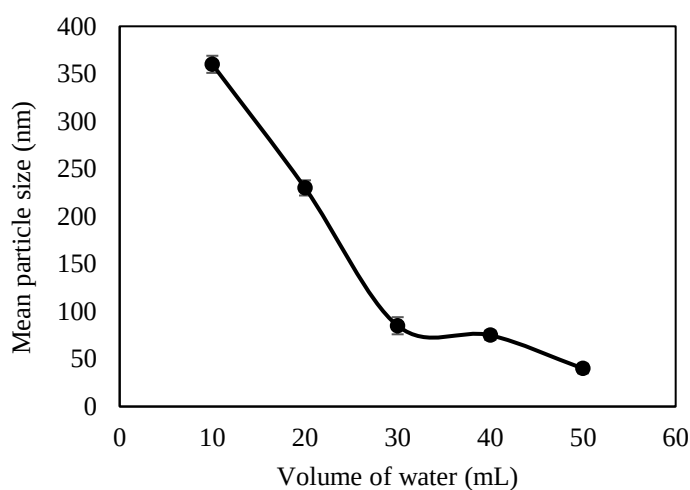


Figure 4.6: Mean Particle Sizes of I-LNPs With Different Volumes of Water (n = 3)

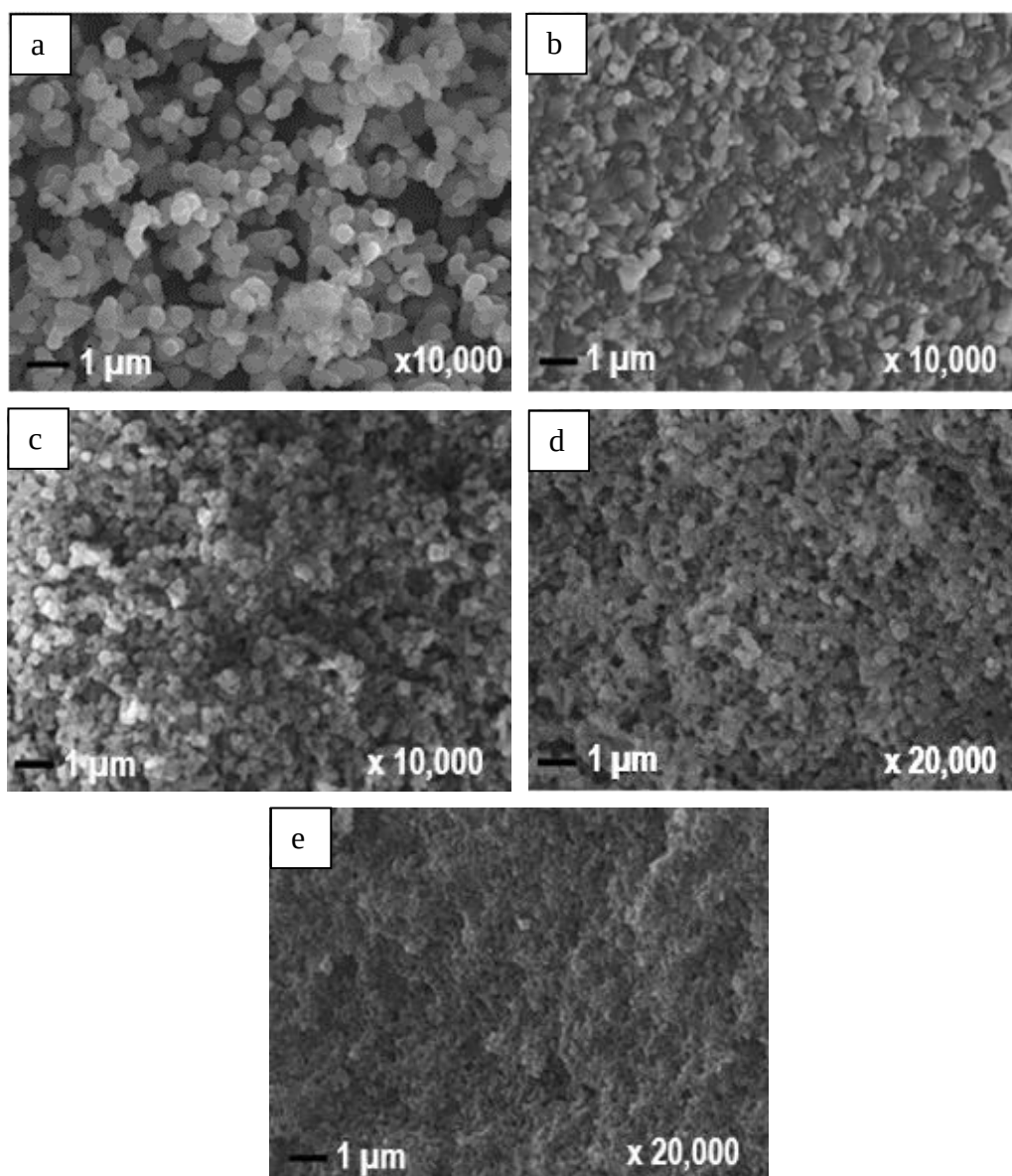


Figure 4.7: SEM Micrographs of I-LNPs After Final Addition of (a) 10 mL, (b) 20 mL, (c) 30 mL, (d) 40 mL and (e) 50 mL of Water

As shown in Figure 4.8, spherical shape of I-LNPs was formed after iodination. This indicated that laccase catalysed iodination did not change the shape of LNPs, and the results were consistent with SEM micrographs in Figure 4.7.

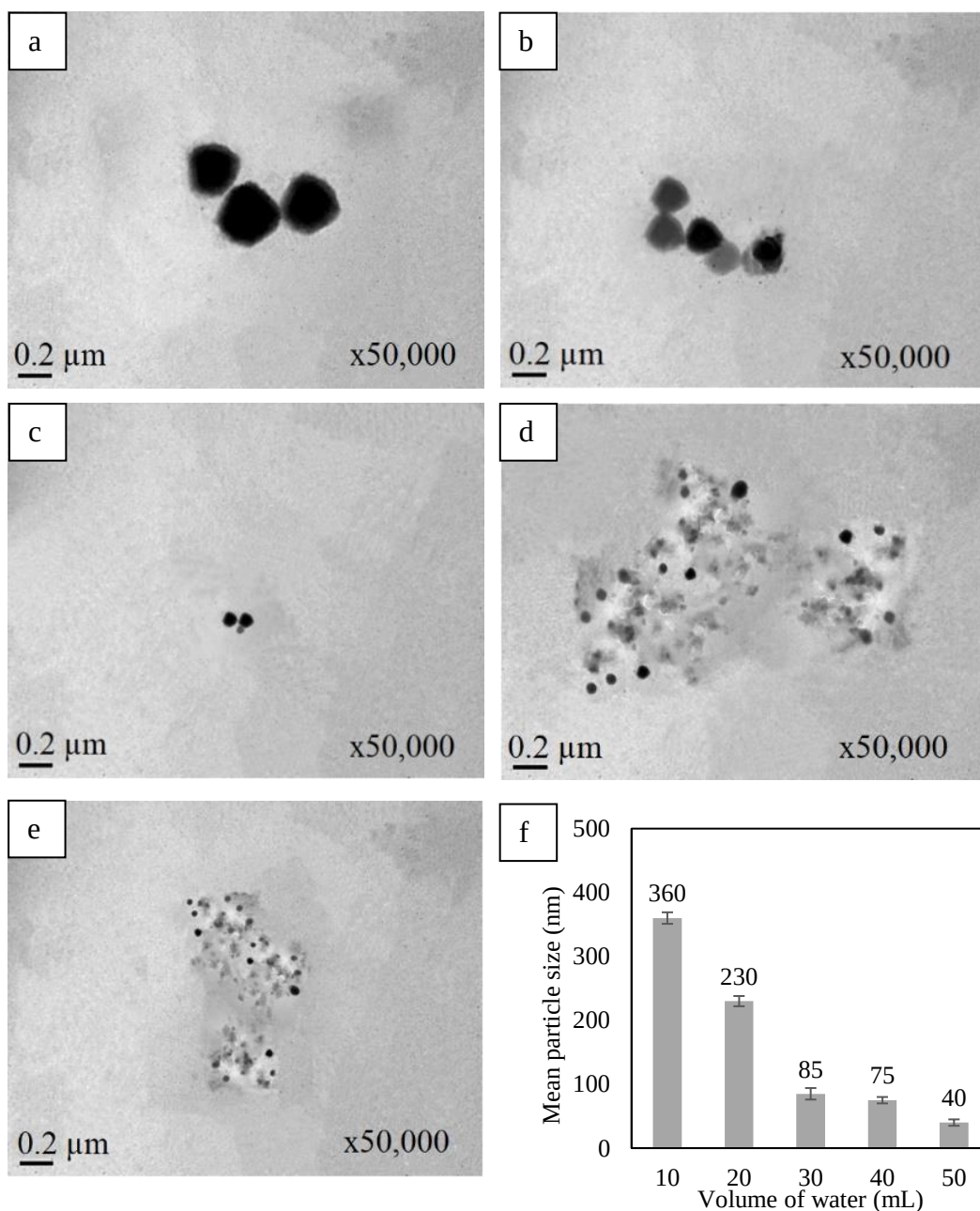


Figure 4.8: TEM Micrographs of I-LNPs After Final Addition of (a) 10 mL, (b) 20 mL, (c) 30 mL, (d) 40 mL, (e) 50 mL of Water and (f) Particle Sizes Distribution of I-LNPs

4.5 EDX Analysis of LNPs and I-LNPs

To confirm the elemental composition of the nanoparticles, the presence of elemental iodine in lignin-based nanoparticles was confirmed by EDX elemental analysis which is shown in Figure 4.9. The EDX spectrum for iodolignin nanoparticles indicated the presence of elements such as carbon, oxygen and iodine.

The peaks detected around 3.4, 4.01, 4.5 KeV, were corresponded to elemental iodine (Dai et al., 2018), with the peak intensities being the highest for carbon, iodine, followed by oxygen. The peak intensity indicated the relative concentration of each element and the amount content of iodine attached to the surface of lignin nanoparticles.

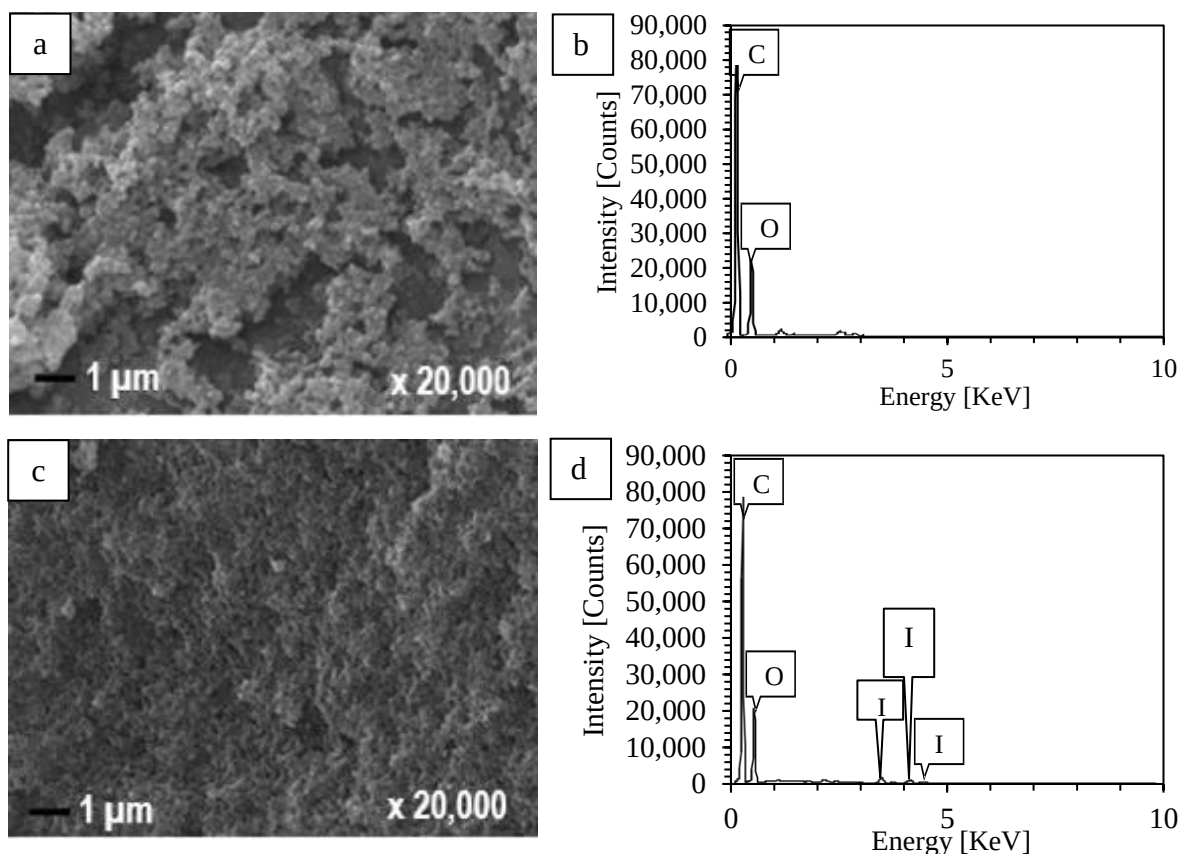


Figure 4.9: The Morphology of (a) Lignin Nanoparticles and (b) its EDX Spectrum, (c) Iodo-Lignin Nanoparticles and (d) its EDX Spectrum

4.6 Dispersion stability of LNPs and I-LNPs

The stability of LNPs and I-LNPs dispersion was evaluated as a function of time and medium conditions. According to Ajiboye et al. (2022), the agglomeration or settling of nanoparticles can reduce the efficacy of fungicide. Therefore, the stability of the nanoparticle was observed in terms of particle size in water dispersions.

Interestingly, both LNPs and I-LNPs showed no specific aggregation and increased of particle size after 90 days (Figure 4.11). As shown in Figure 4.10, the size of I-LNPs at 10:1 (v/v) was slightly increased from 350 nm to 380 nm in water dispersion. This was probably due to a larger particle size of I-LNPs at 10:1 (v/v), which could form aggregation and increased of particle size in water dispersions (Matsakas et al., 2020; Richter et al., 2016). According to Manisekaran et al. (2022), LNPs demonstrates excellent colloidal stability with particle size of less than 200 nm. They concluded that the prepared LNPs with a particle size of less than 200 nm shows no specific aggregation in water dispersions after 90 days. Similarly, Ma et al. (2023) has reported that LNPs demonstrates good colloidal stability with a particle size of less than 200 nm after dispersing in acetone-water for 90 days.

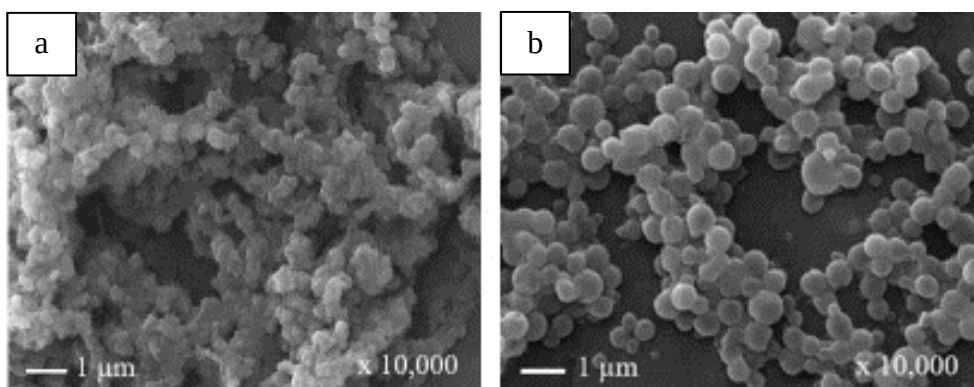


Figure 4.10: SEM Micrographs of (a) LNPs and (b) I-LNPs at 10:1 (v/v) in Water: Acetone After 90 Days of Dispersion

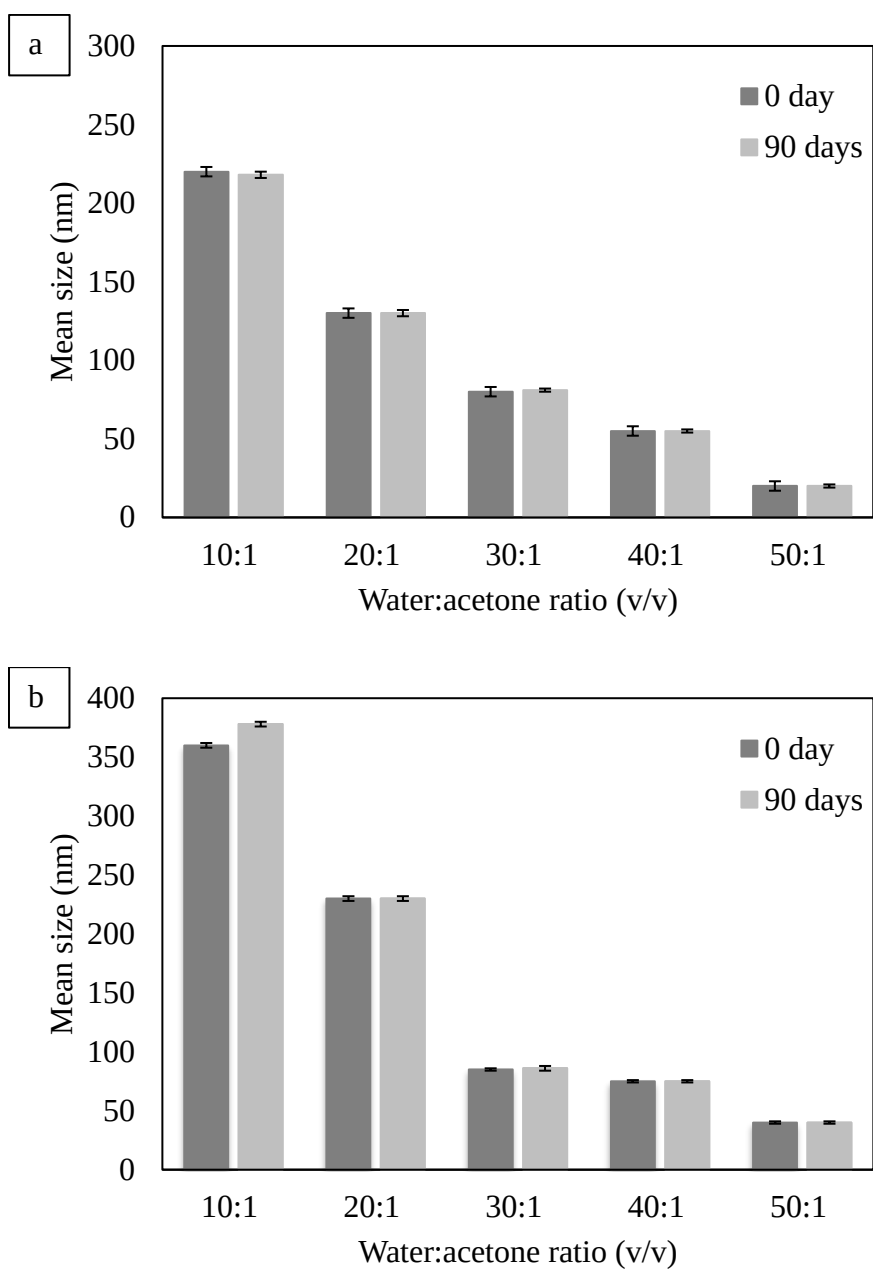


Figure 4.11: Stability of (a) LNPs and (b) I-LNPs by Measuring Mean Particle Sizes in Different Volumes of Water:Acetone as a Function of Time

4.7 Antifungal Activity

The mycelium growth inhibition of different sizes and concentrations were analysed on solid agar media. The growth of the colony was measured, and the growth of inhibition was expressed as the percentage of inhibition. The antifungal activity of lignin nanoparticles was evaluated with different nanosizes ranging from 20 nm to 200 nm. Also, various concentrations ranged from 3mM to 12 mM of LNPs were tested against selected plant pathogenic fungi as shown in Table 4.1.

When the smallest size LNPs at 20 nm was applied as treatment, the mycelium growth inhibitions were significantly reduced. Also, in combination with highest concentration of 12 mM, an increased in growth inhibition was observed. LNPs demonstrated good antifungal activity at 75%, 65%, 68% and 62% against *T. piluliferum*, *Cunninghamella* sp., *A. flavus* and *A. niger*, respectively (Figure 4.12).



Figure 4.12: Mycelial Growth Inhibition of Various Plant Pathogenic Fungi After Treated With 20 nm and 12 mM LNPs

On the other hand, a lower growth of inhibition was observed at 3, 6 and 9 mM with LNPs of 20 nm. According to Gordobil et al. (2018), guaiacyl and syringyl structures play a significant role in determining the antifungal efficacy of various microorganisms. Alkali lignin was reported to have guaiacyl and syringyl structures (Schneider et al., 2021). Therefore, the higher inhibition of LNPs was due to the presence of alkali lignin's low carbohydrate and sulphur derivative.

Comparatively, *F. equiseti* and *P. chermesinum* exhibited low growth inhibition at 38% and 48%, respectively, even when subjected to the highest concentration and smallest nanoparticle sizes. *Fusarium* species are frequently associated with the infection of plant crops, leading to the wilting of crops, particularly on tomato plants. According to Lozovaya et al. (2006) as cited by Ferrari et al. (2021), *Fusarium* and *Penicillium* species have been found to produce lignin peroxidase enzymes. These enzymes are capable of degrading natural lignin, leading to the development of widespread wilt in plants (Chitwood-Brown et al., 2021; Zhang et al., 2022). The degradation of lignin was associated with oxidative process of carbon-carbon and ether linkage units in lignin (You et al., 2019; Weng et al., 2021). Therefore, LNPs demonstrate low growth inhibition at 12 mM against all tested fungi.

To conclude, the size of nanoparticles and concentration of LNPs are important parameters for antifungal studies. High concentration and small nanoparticle diameter of LNPs led to a decrease in the percentage of inhibitions. Also, the combination treatment of 20 nm and 12 mM concentration of LNPs demonstrate good inhibition against *Cunninghamella* sp., *T. piluliferum*, *A. niger* and *A. flavus*. Meanwhile, low growth of inhibition was observed against *F. equiseti* and *P. chermesinum* with nanosized of 20 nm. Therefore, further functionalisation of lignin-based nanoparticles with other antifungal agent is essential to enhance its antifungal activity.

Table 4.1: Antifungal Activity of LNPs Against Various Plant Pathogenic Fungi (%) (n = 3)

Fungi	Percentage of inhibition (%)					
	Size	220 ±5 nm	130 ± 5 nm	80 ±8 nm	55± 5 nm	20± 8 nm
	Conc. (mM)					
<i>F. equiseti</i>	3	15.2 ± 1.3*	16.2 ± 1.3*	17.6 ± 1.3*	19.2 ± 1.3**	20.3 ± 1.3**
	6	16.1 ± 1.3*	18.9 ± 1.5*	20.4 ± 2.0*	23.2 ± 1.5**	25.4 ± 1.5**
	9	18.3 ± 1.0*	20.3 ± 1.0*	25.3 ± 3.0*	28.1 ± 3.0**	30.5 ± 5.0**
	12	24.3 ± 1.5*	26.2 ± 1.5*	28.5 ± 5.0*	35.2 ± 1.5**	43.2 ± 1.3**
<i>A. niger</i>	3	13.4 ± 2.0*	18.3 ± 2.3*	20.7 ± 5.0*	25.2 ± 3.3*	28.3 ± 1.3*
	6	30.1 ± 1.1**	31.2 ± 2.0**	33.6 ± 1.2*	38.4 ± 3.0*	42.6 ± 1.3*
	9	38.2 ± 2.0*	40.3 ± 2.5*	43.3 ± 1.3*	50.6 ± 5.0*	53.3 ± 1.0*
	12	35.1 ± 1.3*	38.4 ± 1.3*	48.5 ± 3.3*	55.2 ± 5.0*	62.6 ± 2.3*
<i>A. flavus</i>	3	18.6 ± 1.3*	23.7 ± 1.3*	28.4 ± 1.3*	30.2 ± 1.3*	37.2 ± 1.3*
	6	37.6 ± 1.0**	38.7 ± 1.3**	40.2 ± 1.0*	43.3 ± 5.3*	40.5 ± 1.0*
	9	51.2 ± 2.0*	48.4 ± 2.0*	46.3 ± 1.5*	45.2 ± 2.1*	48.7 ± 1.3*
	12	53.7 ± 1.0*	55.2 ± 1.3*	58.3 ± 1.3*	67.3 ± 3.5*	68.3 ± 1.5*

Table 4.1 continued

<i>Cunnighamella</i> sp.	3	20.1 ± 1.3*	25.1 ± 1.3*	28.5 ± 1.3*	30.7 ± 1.0*	35.1 ± 1.3*
	6	38.6 ± 2.0*	40.5 ± 1.5**	41.4 ± 1.3**	45.2 ± 1.3*	48.3 ± 2.0*
	9	51.3 ± 1.0*	54.8 ± 3.3*	53.6 ± 1.0*	56.5 ± 3.0*	60.7 ± 2.3*
	12	53.3 ± 2.3*	55.2 ± 1.5*	58.8 ± 2.3*	60.3 ± 3.3*	65.3 ± 3.0*
<i>T. piluliferum</i>	3	21.3 ± 1.3*	23.4 ± 1.3*	25.1 ± 1.3*	30.4 ± 1.3*	40.7 ± 1.3*
	6	33.3 ± 2.1**	35.3 ± 1.0**	40.3 ± 3.0*	45.2 ± 1.5*	53.3 ± 3.3*
	9	45.6 ± 1.3*	53.5 ± 2.3*	56.2 ± 1.3*	58.3 ± 2.3*	63.3 ± 1.2*
	12	63.4 ± 2.3*	65.7 ± 1.0*	68.7 ± 1.0*	70.7 ± 2.7*	75.7 ± 1*
<i>P. chermesinum</i>	3	15.0 ± 1.3*	17.2 ± 1.3*	19.2 ± 1.0*	23.3 ± 1.3**	24.3 ± 5.3**
	6	23.4 ± 1.3*	25.1 ± 1.8*	27.1 ± 3.0*	30.1 ± 2.3**	33.4 ± 1.0**
	9	31.2 ± 3.0*	32.5 ± 2.7*	35.1 ± 1.0*	36.6 ± 1.0**	40.3 ± 1.3**
	12	40.5 ± 1.3**	41.5 ± 3.3**	45.5 ± 2.3*	48.3 ± 1.3**	53.1 ± 3.3**

Data are given as mean ± standard deviation; n = 3 individual experiments. Significant inhibition growth compared to the control is marked by *p<0.05 and **p<0.001 (Tukey's test).

The antifungal activity was further evaluated using iodo-lignin nanoparticles (I-LNPs) on solid agar media. The prepared I-LNPs by laccase catalysed iodination shows enhanced antifungal activity against pathogenic fungi. It has been shown that increasing concentrations and decreasing particle sizes of I-LNPs result in higher antifungal activity for *T. piluliferum*, *A. niger*, *A. flavus*, *F. equiseti*, *Cunninghamella* species, and *P. chermesinum* (Table 4.2). The fungi growth were inhibited by at least 90% with nanosized of 40 nm and 12 mM concentration (Figure 4.13). Apart from that, moderate antifungal activity (50% to 70%) was observed at 3 mM to 6 mM concentrations. Therefore, for inhibition of all fungi species, I-LNPs at the 12 mM concentration could be used.

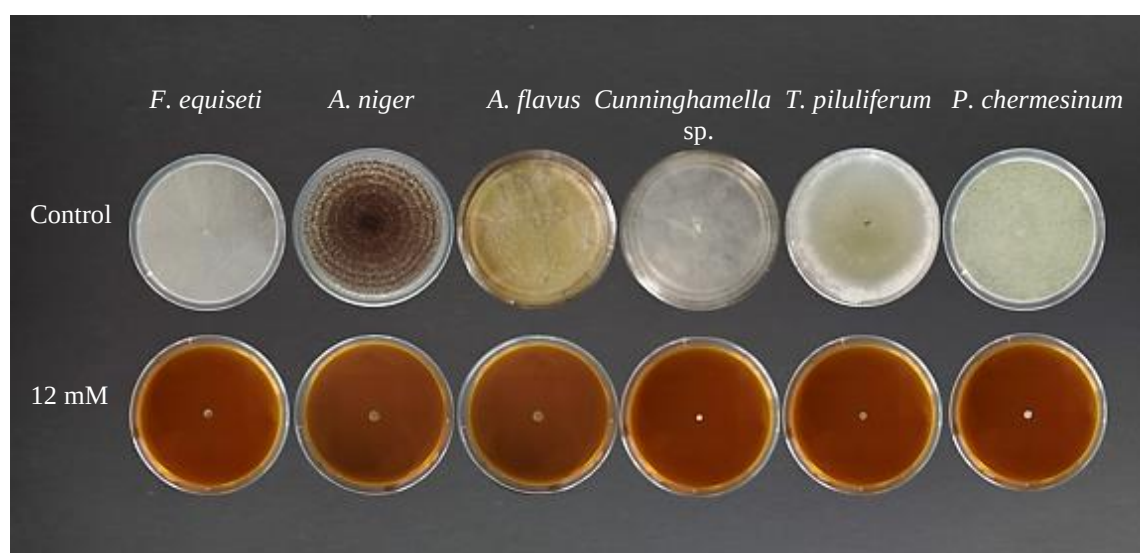


Figure 4.13: Mycelial Growth Inhibition of Various Plant Pathogenic Fungi After Treated With 40 nm and 12 mM I-LNPs

These results agree with other author which reported that iodine compound has good antifungal activity at millimolar concentration against plant pathogenic fungi. According to Nefzi et al. (2019), treatment of iodine could effectively reduce the growth of *Fusarium*,

Penicillium and *Aspergillus* species on tomato plants at 90% inhibition using 2% iodine or equivalent to 0.08 M of iodine solution. Similarly, Riyazuddin et al. (2021) reported that 20 millimolar of aqueous iodine solutions exhibit excellent antifungal activity against *Fusarium*, *Aspergillus* and *Cunninghamella* species. Comparatively, iodine-based compound sustains a slow release of elemental iodine. The biocidal effect of iodine-based compound was probably due to synergy effect of iodine and its complex (Golubkina et al., 2020; Borjihan et al., 2023; Long et al., 2023).

The biocidal mechanisms of iodine were associated with the release of membrane-associated toxicity effect and the release of elemental iodine from iodine-based compound (Chang et al., 2020). For instance, iodine will first rupture the cell wall of fungi, resulting in leakage and shrinking of the cytoplasmic structure. Finally, the coagulation of proteins and precipitation of mineral crystals occurs due to dehydration of the cytoplasm (McDonnell et al., 1999). Similarly, the treatment of I-LNPs increased the lipophilicity of fungi cell wall due to the release of free elemental iodine.

To conclude, the application of I-LNPs as antifungal agent has led to a significant reduction in the mycelial growth of fungi. Also, I-LNPs demonstrates the highest antifungal efficacy when applied at nanosize of 40 nm and 12 mM concentration. Thus, I-LNPs can be used as a promising antifungal agent against various plant pathogenic fungi.

Table 4.2: Antifungal Activity of I-LNPs Against Various Plant Pathogenic Fungi (%) (n = 3)

Fungi	I-LNPs size					
	Size	360 ±5	230 ± 5 nm	85 ±8 nm	75± 5 nm	40± 8 nm
	Conc. (mM)					
<i>F. equiseti</i>	3	50.6 ± 2.3*	53.3 ± 2.5*	59.4 ± 1.3*	62.8 ± 1.3*	75.3 ± 1.3*
	6	70.4 ± 3.0*	73.7 ± 2.0*	73.3 ±1.3*	83.3 ± 1.0*	81.4 ± 1.3*
	9	89.4 ± 2.3*	93.4 ± 2.3*	93.3 ± 2.3*	95.2 ± 2.3*	95.2 ± 3.1*
	12	93.3 ± 1.0*	95.3 ± 1.0*	98.2 ± 1.0*	98.7 ± 1.3*	98.7 ± 1.3*
<i>A. niger</i>	3	35.3 ± 2.3*	40.3 ± 1.5*	45.5 ± 1.3*	50.7 ± 1.3*	59.7 ± 1.3*
	6	48.3 ± 1.0*	50.1 ± 3.0*	61.2 ± 1.0*	64.1 ± 1.5**	65.4 ± 1.3**
	9	63.4 ± 3.3*	66.2 ± 1.0*	67.1 ± 5.0*	71.5 ± 5.0*	78.3 ± 3.3*
	12	80.4 ± 2.0*	84.3 ± 2.0*	86.7 ± 1.3*	89.6 ± 1.3*	95.6 ± 2.3*
<i>A. flavus</i>	3	38.8 ± 2.3*	43.3 ± 1.3*	46.2 ± 1.5*	48.5 ± 2.3*	53.7 ± 1.5*
	6	48.3 ± 1.0*	53.8 ± 2.0*	55.2 ± 3.3*	58.3 ± 1.3*	63.9 ± 2.3*
	9	60.4 ± 3.3*	63.5 ± 1.0*	65.4 ± 1.3*	66.4 ± 1.2*	70.4 ±1.3*
	12	80.7 ± 2.0*	83.6 ± 5.0*	85.6 ± 1.3*	86.3 ± 2.0*	95.3 ± 3.3*

Table 4.2 continued

<i>Cunninghamella</i> sp.	3	48.5 ± 1.3*	55.6 ± 2.3*	58.3 ± 1.0*	60.5 ± 1.0*	65.4 ± 1.5*
	6	64.3 ± 1.3*	66.3 ± 2.0*	67.6 ± 2.0*	70.7 ± 1.0*	75.1 ± 2.0*
	9	78.3 ± 2.0*	81.4 ± 1.0*	83.2 ± 1.0*	83.3 ± 1.0*	83.1 ± 3.0*
	12	81.2 ± 3.0*	85.3 ± 3.3*	86.2 ± 5.0*	89.3 ± 1.0*	93.3 ± 3.0*
<i>T. piluliferum</i>	3	55.3 ± 1.3*	58.6 ± 1.3*	60.3 ± 2.3*	68.0 ± 1.3*	75.4 ± 2.3*
	6	68.8 ± 1.3*	73.3 ± 1.3*	75.7 ± 1.3*	72.4 ± 1.0*	85.2 ± 1.0*
	9	78.2 ± 5.0*	82.3 ± 3.3*	86.2 ± 2.0*	93.2 ± 1.0*	92.5 ± 1.3*
	12	83.6 ± 2.0*	88.5 ± 1.2*	93.4 ± 1.2*	96.6 ± 1.0*	98.7 ± 1.3*
<i>P. chermesinum</i>	3	40.2 ± 1.0*	55.7 ± 1.5*	65.3 ± 1.0*	68.3 ± 1.5*	70.3 ± 1.0*
	6	68.3 ± 1.3*	71.2 ± 3.0*	73.3 ± 2.0**	74.9 ± 1.0**	83.1 ± 1.0*
	9	81.1 ± 1.0*	84.2 ± 1.0*	90.3 ± 1.0*	92.8 ± 3.0*	90.2 ± 2.0*
	12	85.5 ± 1.5*	90.4 ± 1.0*	93.9 ± 1.0*	95.2 ± 2.0*	98.6 ± 1.0*

Data are given as mean ± standard deviation; n = 3 individual experiments. Significant inhibition growth compared to the control is marked by *p<0.05 and **p<0.001 (Tukey's test).

4.8 Surface Morphology of Bamboo

4.8.1 SEM Analysis of Bamboo

Figure 4.14 (e) shows a smooth surface of bamboo cell walls. Following treatment, a crusty layer of LNPs was seen covering a bamboo cell wall surface (Figure 4.14 (a)). Also, I-LNPs layer was formed on the surface of bamboo after laccase treatment. Similarly, a crusty layer of lignin forming on wood surface was reported by Zikeli et al. (2019). After leaching with water, only a small number of nanospheres that had kept their original form could be seen on a background of flaky and crusty aggregates with a mostly smooth surface.

The formation of iodine on bamboo was likely due to the substitution of oxygen-containing groups which were formed from laccase catalysed oxidation (Prosper et al., 2018). In the case of lignin, laccase could cleave the bonds between C_{α} - C_{β} cleavage, and alkyl-aryl cleavage (Kawai et al., 1999; Zheng et al., 2019). The electron oxidation formed free radicals known as phenoxy radicals and bind with elemental iodine (Panda & Duarte-Sierra, 2022). Similarly, laccase mediated systems could depolymerise lignin and functionalised lignin with iodine.

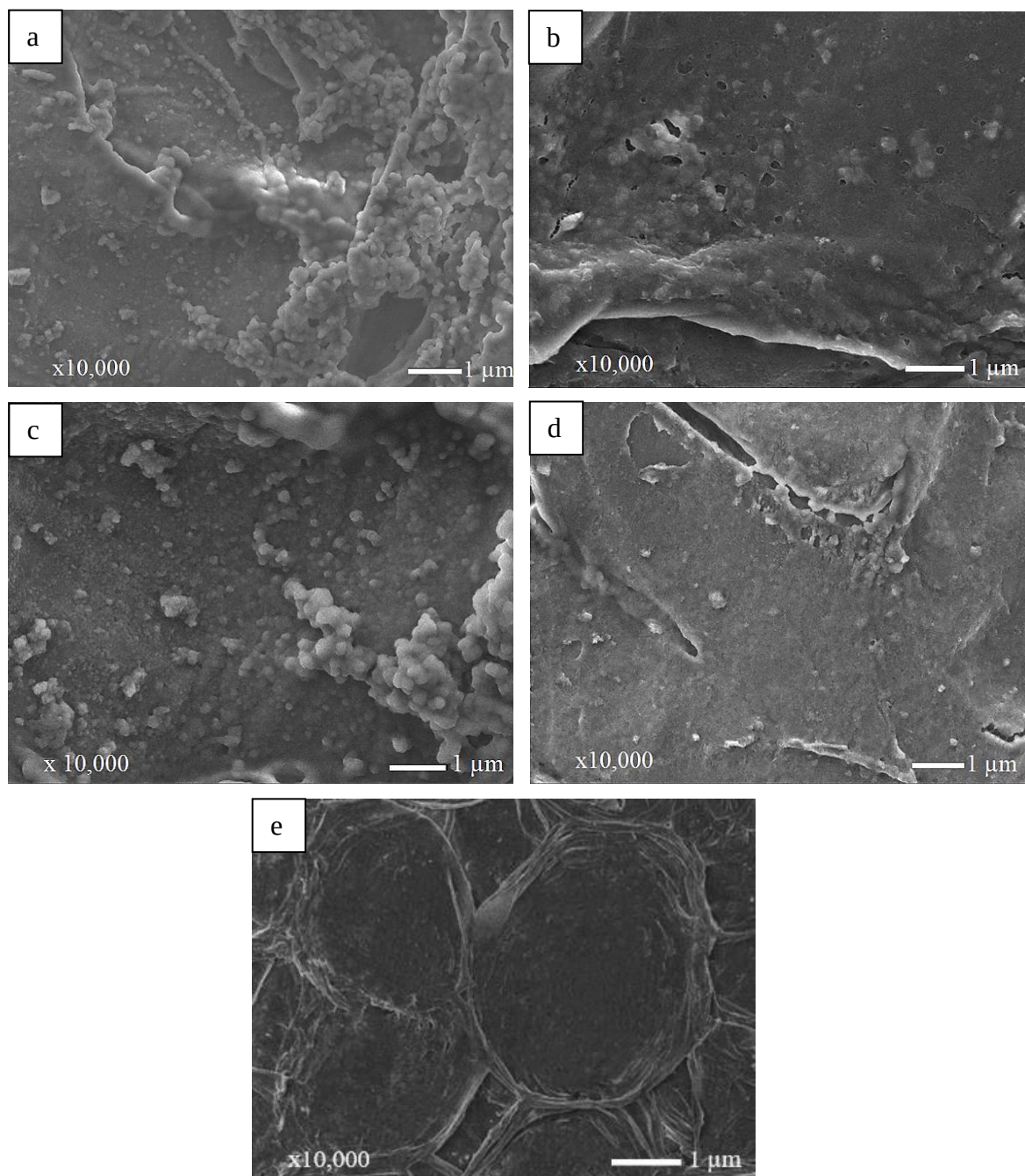


Figure 4.14: SEM Micrographs of (a) LNPs Treated Bamboo (b) After Leaching, (c) I-LNPs Treated Bamboo (d) After Leaching and (e) Untreated Bamboo

4.8.2 EDX Analysis of Bamboo

EDX analysis of LNP-treated bamboo shows the presence of a relatively small amount of sulphur (0.8%) on the surface of the bamboo (Figure 4.15 (b)). The presence of sulphur was most probably originated from the commercialised alkali lignin (Iravani & Varma, 2020). As shown on Figure 4.15 (d), the presence of elemental iodine (11.8%) was formed on the surface of bamboo. Hence, the strategy of incorporating iodine onto the surface of bamboo can be achieved through the utilisation of laccase catalysed iodination.

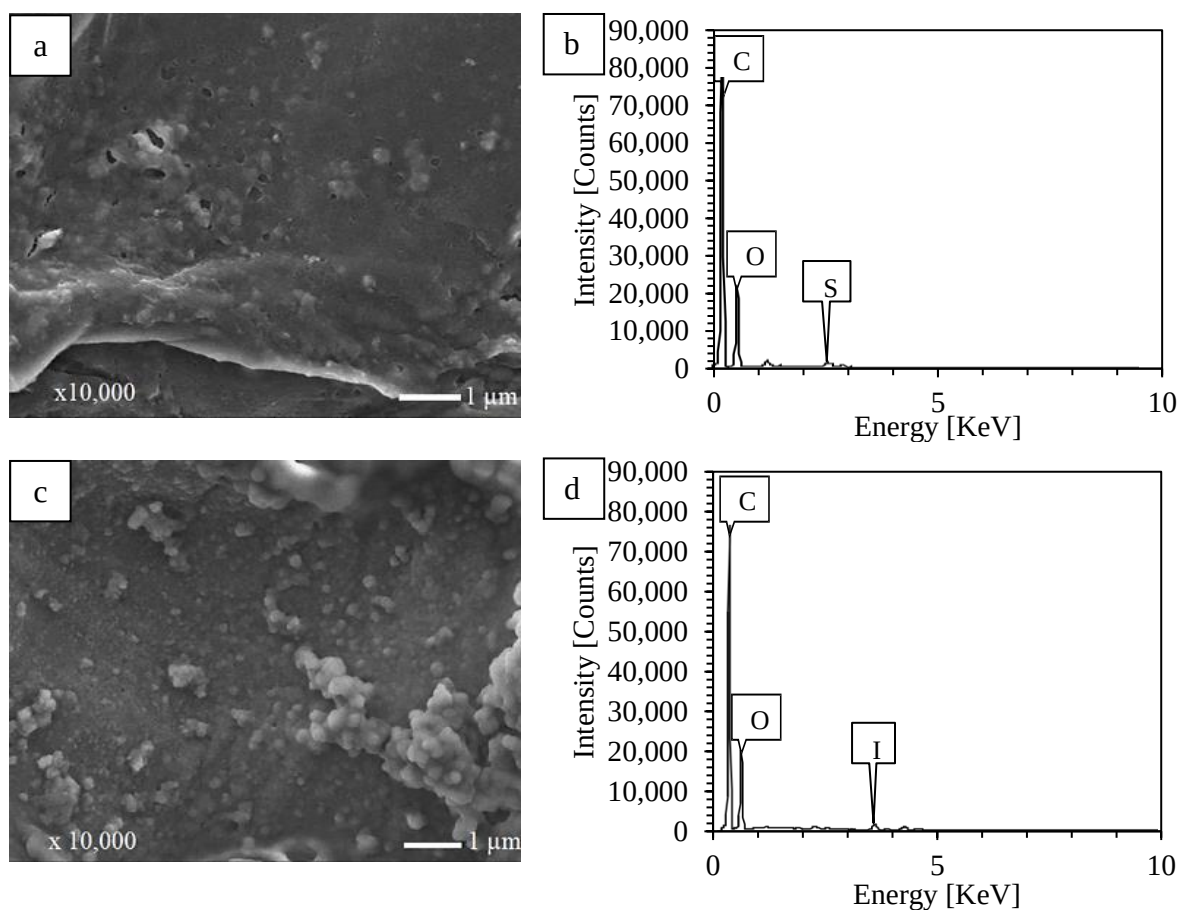


Figure 4.15: SEM Micrographs of (a) LNPs Treated Bamboo and (b) its EDX Spectrum, (c) I-LNPs Treated Bamboo and (d) its EDX Spectrum

4.9 Identification of Fungi

4.9.1 Macroscopic and Microscopic Identification

The identification of macroscopic characteristics of the 3 fungal isolates from bamboo was conducted after the isolation process was completed. Generally, the fungal isolates were characterised by observing the front plate views on the basis of their nature of growth, colour, shape, form, margin, and surface texture. The microscopic observations of fungal isolates were seen under compound light microscope.

The isolation of fungi from the surface of bamboo culm of *Dendrocalamus asper* resulted in 3 morphologically distinct fungi abbreviated as B1, W1 and O1 (Figure 4.16). The three fungi also produced spores that were different in shape and size when observed under compound light microscope after staining with lactophenol cotton blue. Isolate B1 formed slow growing, powdery colonies with greenish-blue or blue-green coloration. The fungus produces septate hyphae and elongates with branched conidiophores that bear oval or cylindrical conidia. The conidiophores were predominantly bi-verticillate with broom-like structures having round conidiophores and sporadically smooth (Houbraken et al., 2011)

Isolate W1 on the other hand grows very rapidly producing hyphae that form fluffy masses with various shades of white that slowly turns grey with time. Under the microscope, the fungus was observed to produce sporangia at the tips of sporangiophores which contain sporangiospores. Similar with isolate W1, fungal isolate O1 was also a fast-growing fungus that reached full plate within just 7 days of growth in 9 cm Petri dish. The fungus was first grown with white coloured mycelium which then slowly formed green coloured ring like pattern. When visualised under the microscope, the fungus was observed to produce branches of septate hyphae and conidiophore that harbour spores on the tip of the branch.

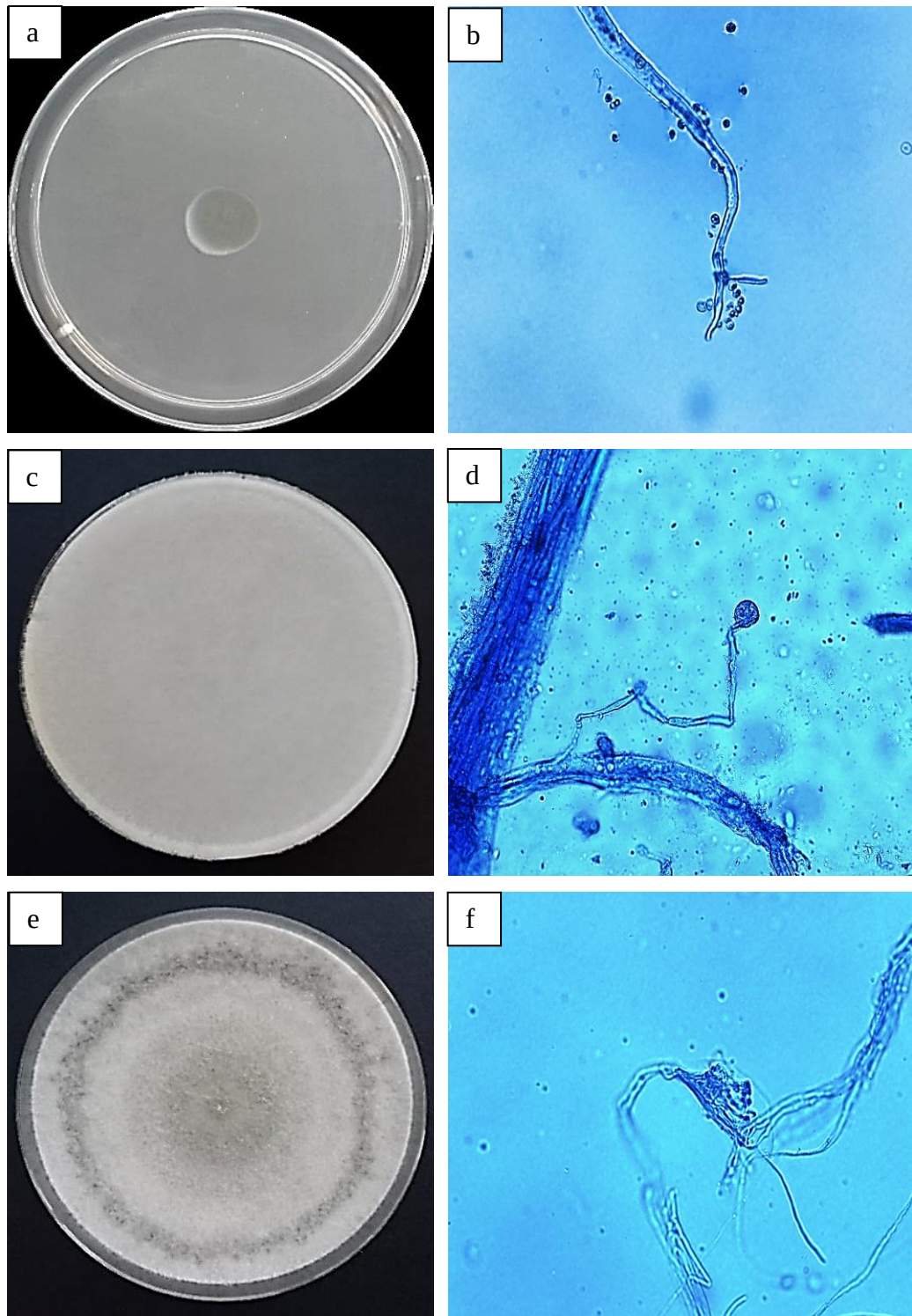


Figure 4.16: Macroscopic Images of (a) B1, (c) W1, and (e) O1 and Microscopic Images of (b) B1, (d) W1 and (f) O1 at 400x Magnification Under Compound Light Microscope

4.9.2 Molecular Identification

The isolated fungi from bamboo were identified molecularly for further antifungal test on bamboo. Further PCR amplification of the extracted fungal DNA produced PCR product that was about 650 bp in size for all the 3 fungal isolates (Figure 4.17). Further sequencing and BLAST analysis with NCBI GenBank shows that isolates B1 shared 99% sequence similarity with *Penicillium sumatrense*, isolate W1 with 98% sequence similarity with *Cunninghamella* sp., while O1 shared 100% sequence similarity with *Pleosporales* sp.

These 3 isolates were known plant pathogens that have been well documented in literature, especially *Cunninghamella* species. The species of *Cunninghamella* such as *Cunninghamella echinulate* causes soft rot in cucumber (Cherian, 2005), while *Cunninghamella elegans* causes cassava root rot (Pham & Tran, 2021). *P. sumatrense* has been reported to cause blue mold of table grape (*Vitis vinifera* L.) (Mahdian & Zafari, 2017) and cauliflower mushroom (*Sparassis crispa*) (Liu et al., 2018). Similarly, *Pleosporales* sp. has also been found to cause the leaf spot of *Osmanthus fragrans* (Wijesinghe et al., 2020).

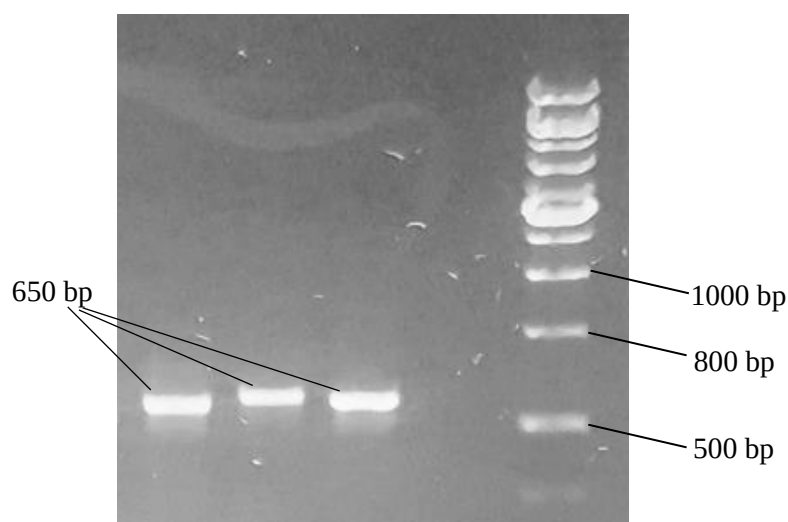


Figure 4.17: Agarose Gel Electrophoresis of (a) B1, (b) W1 and (c) O1 PCR Product of Approximately 650 bp. Lane (d) 1Kb DNA Ladder (Transgen)

4.10 Antifungal Activity of Bamboo

4.10.1 Mould Resistance

The efficacy of LNPs and I-LNPs in protecting lignocellulosic materials of *Dendrocalamus asper* against *P. sumatrense*, *Cunninghamella* sp. and *Pleosporales* sp. was evaluated based on mould growth rating (Figure 4.18). Also, water leaching test was carried out to test the antifungal activity of treated bamboo. Treatment of bamboo with either LNPs or I-LNPs could clearly reduce the fungi growth (Figure 4.19).

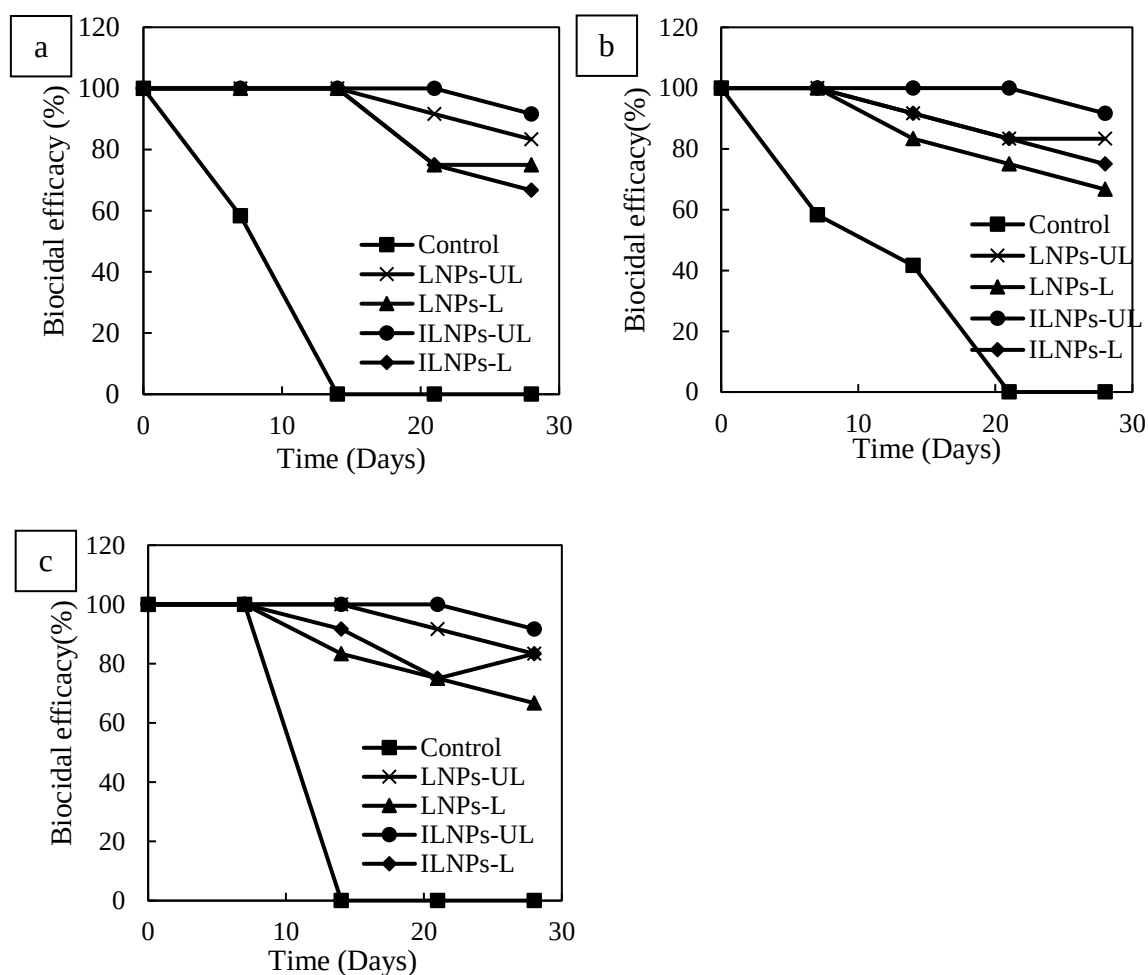


Figure 4.18: Biocidal Efficacy of Unleached and Leached Treated Bamboo Against (a) *P. sumatrense*, (b) *Cunninghamella* sp. and (c) *Pleosporales* sp.

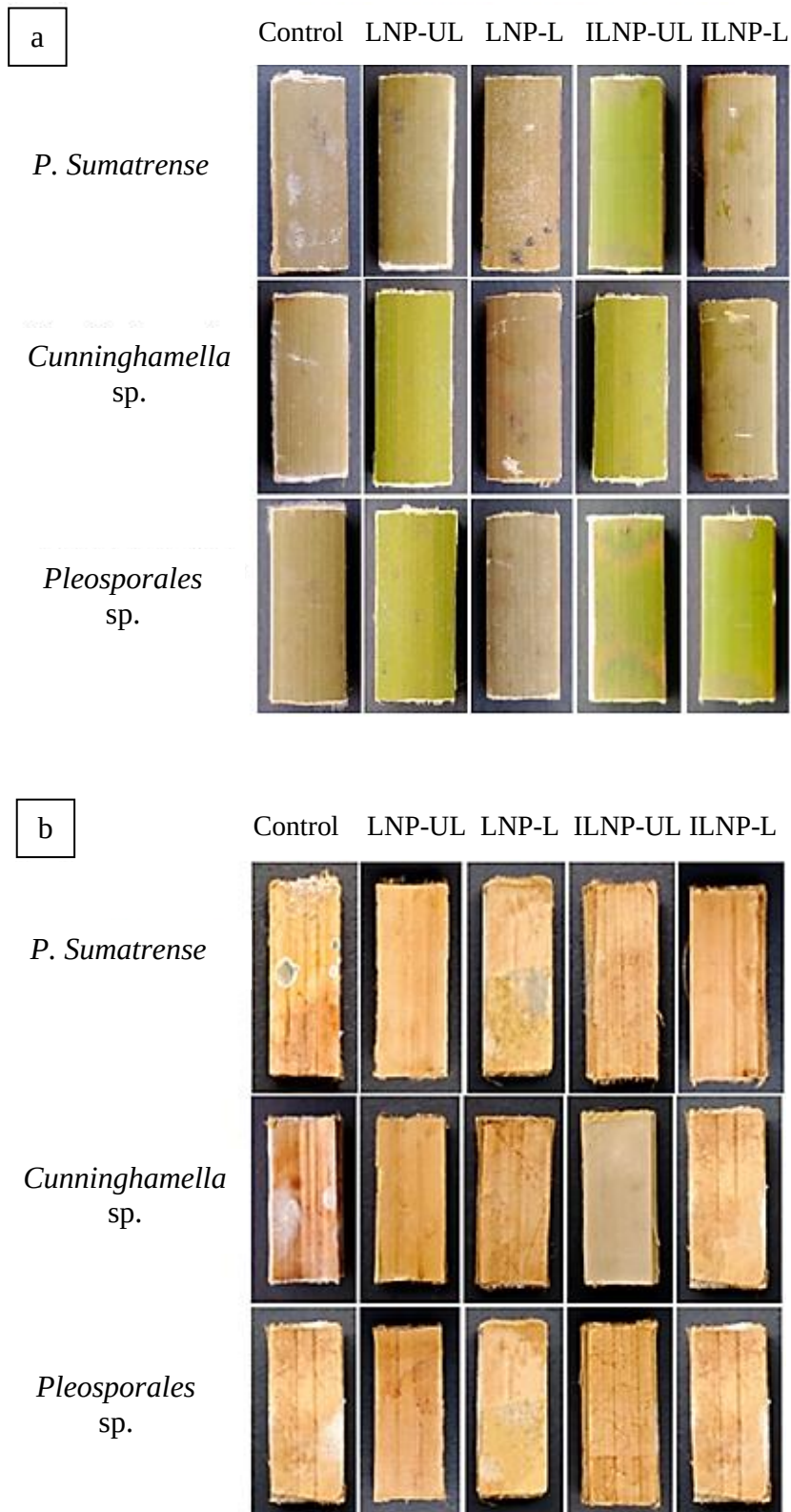


Figure 4.19: Mould Growth on Bamboo Inspected on the (a) Front View and (b) Back View

The bamboo treated with lignin nanoparticles demonstrate a good biocidal efficacy at 80%. However, biocidal effect of lignin nanoparticles (LNPs) was slightly decreased after leaching. The observed bamboo exhibits a lower biocidal efficacy at 65% against *P. sumatrense*, *Cunninghamella* and *Pleosporales* species.

According to Gordobil et al. (2018), the antifungal properties of LNPs were mainly affected by the presence of aromatic compounds structure of lignin such as guaiacyl and syringyl unit, which possessed antifungal activity. However, lignin is weak antifungal activity. Therefore, functionalisation of lignin-based nanoparticles is needed to enhance its antifungal properties.

Interestingly, the biocidal effect was enhanced when I-LNPs was subjected as treatment. The bamboo treated with I-LNPs exhibited excellent efficacy at 90% against *P. sumatrense*, *Cunninghamella* sp., and *Pleosporales* sp (Figure 4.19). Also, I-LNPs retained a good biocidal effect after leaching with water at 66% to 83%. In comparison to another study by Prosper et al. (2018), the biocidal efficacy of iodo-lignin nanoparticles was slightly higher than treatment with iodide alone.

4.10.2 Decay Resistance

The decay resistance of bamboo was determined gravimetrically after 12 weeks of incubations (Figure 4.19). As shown in Table 4.3, LNPs recorded a lower mass loss at less than 8% against *P. sumatrense*, *Cunninghamella* sp. and *Pleosporales* sp. However, the decay resistance was greatly decreased after leaching with water. For instance, LNPs recorded the highest mass loss at 14% against *Cunninghamella* sp. after leaching with water.

According to Ferrari et al. (2021), *Penicillium* and *Cunninghamella* species have been found to produce lignin peroxidase enzymes. These enzymes are capable of degrading natural lignin in wood and bamboo. Therefore, LNPs-block demonstrated a greater mass loss against *Penicillium* and *Cunninghamella* species.

Table 4.3: Mass Loss of Bamboo After 12 weeks of Incubations (n = 3)

Treatment	Mass loss (%)		
Fungi	<i>P. sumatrense</i>	<i>Cunninghamella</i> sp.	<i>Pleosporales</i> sp.
Control	13.8 ± 1.0	22.1 ± 1.0	20.5 ± 3.0
LNPs (unleached)	2.4 ± 0.5	8.5 ± 0.5	3.0 ± 0.1
LNPs (leached)	7.5 ± 1.0	14.8 ± 1.0	7.6 ± 0.1
I-LNPs (unleached)	2.7 ± 1.0	3.7 ± 0.1	1.8 ± 1.0
I-LNPs (leached)	6.5 ± 1.0	5.2 ± 1.0	2.5 ± 0.5

Data are given as mean ± standard deviation.

Comparatively, the bamboo treated with I-LNPs exhibited excellent decay resistance. When I-LNPs was used as a treatment, a lower mass loss at 1% to 3% were observed against *P. sumatrense*, *Cunninghamella* sp. and *Pleosporales* sp. However, the decay resistance was slightly decreased after leaching at 2% to 6%. The enhanced antifungal activity of I-LNPs was probably attributed to the higher antifungal activity of elemental iodine (Tsuda et al., 2020). Thus, the bamboo treated with iodo-lignin nanoparticles is a promising strategy to enhance its decay resistance against decay fungi. Also, bamboo treated with I-LNPs is found to be effective after leaching with water.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In summary, lignin nanoparticles (LNPs) were successfully prepared by solvent shifting method. Lignin-based nanoparticles (I-LNPs) were successfully modified with iodine by laccase catalysed iodination reaction. In relation to the mycelial growth inhibition test, efficient inhibition of LNPs and I-LNPs are verified. Lignin nanoparticles show moderate antifungal activity with a nanosize of 20 nm and 12 mM concentration. In combination with iodine, I-LNPs shows greater antifungal activity against *T. piluliferum*, *Cunninghamella* sp. *A. niger*, *A. flavus*, *F. equiseti* and *P. chermesinum*. The maximum antifungal activity of I-LNPs was achieved using the nanosize of 40 nm at 12 mM concentration. Besides that, I-LNPs exhibit excellent biocidal efficacy than LNPs and untreated bamboo against *P. sumatrense*, *Cunninghamella*, and *Pleosporales* species. Also, the bamboo treated with I-LNPs exhibits enhanced decay resistance than the bamboo treated with LNPs, even after undergoing leaching. Thus, the findings indicate that I-LNPs can be used as a potential antifungal agent for bamboo protections.

5.2 Recommendations

In this work, although I-LNPs has demonstrated remarkable efficacy as an antifungal agent, further research is required to determine their toxicity before its application as a potential nano-fungicide especially on plant crops. The cytotoxicity study on plant is vital to determine the appropriate amount of iodine being absorbed in plant crops. This is crucial to avoid unnecessary side effect on human health. For commercial purposes, further field test

is required to assess its toxicity as a potential antifungal agent on bamboo. LNPs have shown tremendous opportunity for value-added applications. However, most of the preparation methods use hazardous solvents. Therefore, researchers should develop eco-friendly methods to synthesise LNPs. It is essential to use green solvents such as water and room temperature ionic liquids. Meanwhile, the field of micro-sized lignin has been widely investigated for various applications such as drug delivery carriers, antibacterial agents, and fertilisers. The application of LNPs for broader applications still faces some challenges because of the variable structure and polydispersity of lignin. Thus, it is significant to optimise the particle size and stability of LNPs in different conditions such as acidic or alkaline medium. The stability of nanoparticle dispersion is critical in determining the efficacy of LNPs as fungicides or drug delivery carriers.

REFERENCES

- Abreham, B., Tariku, A., Adane, H., Addisalem, N., Fitala, G., Tigist, G., Musin, K., & Admas, B. (2015). Isolation and characterization of efficient cellulolytic fungi from degraded wood and industrial samples. *African Journal of Biotechnology*, 14(48), 3228-3234.
- Abueva, C., Ryu, H. S., Min, J. W., Chung, P. S., You, H. S., Yang, M. S., & Woo, S. H. (2021). Quaternary ammonium N, N, N-trimethyl chitosan derivative and povidone-iodine complex as a potent antiseptic with enhanced wound healing property. *International Journal of Biological Macromolecules*, 182, 1713-1723.
- Acciardo, E., Tabasso, S., Cravotto, G., & Bensaid, S. (2022). Process intensification strategies for lignin valorization. *Chemical Engineering and Processing-Process Intensification*, 171, 108732.
- Afifi, O. A., Tobimatsu, Y., Lam, P. Y., Martin, A. F., Miyamoto, T., Osakabe, Y., Okasabe, K., & Umezawa, T. (2022). Genome-edited rice deficient in two 4-coumarate: coenzyme a ligase gene displays diverse lignin alterations. *Plant Physiology*, 190(4), 2155-2172.
- Ago, M., Tardy, B. L., Wang, L., Guo, J., Khakalo, A., & Rojas, O. J. (2017). Supramolecular assemblies of lignin into nano- and microparticles. *MRS Bulletin*, 42(5), 371-378.
- Ajiboye, T. O., Ajiboye, T. T., Marzouki, R., & Onwudiwe, D. C. (2022). The versatility in the applications of dithiocarbamates. *International Journal of Molecular Sciences*, 23(3), 1317.
- Akpınar, I., Unal, M., & Sar, T. (2021). Potential antifungal effects of silver nanoparticles

- (AgNPs) of different sizes against phytopathogenic *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) strains. *SN Applied Sciences*, 3(4), 506.
- Alegbeleye, O., Odeyemi, O. A., Strateva, M., & Stratev, D. (2022). Microbial spoilage of vegetables, fruits and cereals. *Applied Food Research*, 2(1), 100122.
- Ali, M. R., Abdullah, U. H., Ashaari, Z., Hamid, N. H., & Hua, L. S. (2021). Hydrothermal modification of wood: A review. *Polymers*, 13(16), 2612.
- Alvindia, D. G. (2018). The antagonistic action of *Trichoderma harzianum* strain DGA01 against anthracnose-causing pathogen in mango cv. 'Carabao.' *Biocontrol Science and Technology*, 28(6), 591–602.
- Ando, D., & Ralph, J. (2019). Method to regioselectively iodine-tag free-phenolic aromatic end-groups in lignin for ¹H-¹³C-HSQC NMR analysis. *ACS Sustainable Chemistry and Engineering*, 7(22), 18624–18629.
- Andriulo, F., Vespignani, L., Steindal, C. C., Bortolini, M., & de Ferri, L. (2022). Evaluation of sol-gel hybrid nanocomposites for dry medieval wood. *Journal of Cultural Heritage*, 56, 96-107.
- ASTM D4445-10 standard. (2019). Standard Test Method for Controlling Sapstain and Mould on Unseasoned Lumber (Laboratory Method). West Conshohocken, USA: Publish America.
- AWPA E-10 standard. (2020). American Wood Protection Association 2020 Book of Standards. Birmingham, USA: American Wood Protection Association.
- Baboo, D., Biswas, S. K., Singh, D. R., Jatav, A. L., & Singh, R. (2021). Effect of inorganic chemicals as inducer on the growth parameters of potato (*Solanum tuberosum* L.) against common scab disease caused by *Streptomyces scabies* (Thaxter) Waksman & Henrichi. *Journal of Pharmacognosy and Phytochemistry*, 10(2), 1287-1291.

- Behboudi, G., Shayesteh, K., Yarak, M. T., Ebrahimi, H. A., & Moradi, S. (2021). Optimized synthesis of lignin sulfonate nanoparticles by solvent shifting method and their application for adsorptive removal of dye pollutant. *Chemosphere*, 285, 131576.
- Bi, Z., Morrell, J. J., Lei, Y., Yan, L., & Ji, M. (2022). Eco-friendly and mildly modification of wood cell walls with heat treated wood extracts to improve wood decay resistance. *Industrial Crops and Products*, 184, 115079.
- Borjihan, Q., Meng, S., Bai, H., Chen, T., Hu, X., Xiao, D., Shi, L., & Dong, A. (2023). Active iodine regulated in cow dung biochar-based hydrogel combined with PDT/PTT for MRSA infected wound therapy. *Materials & Design*, 231, 112051.
- Budnyak, T. M., Slabon, A., & Sipponen, M. H. (2020). Lignin-inorganic interfaces: Chemistry and applications from adsorbents to catalysts and energy storage materials. *ChemSusChem*, 13(17), 4344-4355.
- Burks, C., Darby, A., Gómez Londoño, L., Momany, M., & Brewer, M. T. (2021). Azole-resistant *Aspergillus fumigatus* in the environment: Identifying key reservoirs and hotspots of antifungal resistance. *PLoS Pathogens*, 17(7), e1009711.
- Cailotto, S., Gigli, M., Bonini, M., Rigoni, F., & Crestini, C. (2020). Sustainable strategies in the synthesis of lignin nanoparticles for the release of active compounds: a comparison. *ChemSusChem*, 13(17), 4759-4767.
- Caracciolo, P. C., Abraham, G. A., Battaglia, E. S., & Bongiovanni Abel, S. (2023). Recent progress and trends in the development of electrospun and 3d printed polymeric-based materials to overcome Antimicrobial Resistance (AMR). *Pharmaceutics*, 15(7), 1964.
- Chairat, M., & Wisessombat, S. (2021). Dyeing of silk yarn with the natural extract from the underutilized tropical fruit pericarp of *Ptychosperma macarthurii*. *The Journal of The Textile Institute*, 112(2), 207-215.

- Chandel, P. K., Prajapati, R. K., & Dhurwe, R. K. (2018). Documentation of post-harvest methods and value addition of different NTFPs in Dhamtari forest area. *Journal of Pharmacognosy and Phytochemistry*, 7(1), 1518-1523.
- Chang, J., Zhao, G., Zhao, X., He, C., Pang, S., & Shreeve, J. N. M. (2020). New promises from an old friend: Iodine-rich compounds as prospective energetic biocidal agent. *Accounts of Chemical Research*, 54(2), 332-343.
- Chaowana, K., Wisadsatorn, S., & Chaowana, P. (2021). Bamboo as a sustainable building material-culm characteristics and properties. *Sustainability*, 13(13), 7376.
- Chen, M., Ye, L., Semple, K., Ma, J., Zhang, J., Weng, Y., Yan, H., Fei, B., & Dai, C. (2022). A new protocol for rapid assessment of bond durability of bio-based pipes: Bamboo winding composite pipe as a case study. *European Journal of Wood and Wood Products*, 80(4), 947-959.
- Chen, Z., Du, K., Yin, H., Zhang, D., Gao, J., Song, W., & Zhang, S. (2023). Surface modification of bamboo fiber with dopamine associated by laccase for poly (3-hydroxybutyrate) biocomposites. *Journal of Cleaner Production*, 389, 135996.
- Cherian, T. T. (2005). Soft rot of cucumber (*Cucumis sativus* L.) by *Cunninghamella echinulata* Thaxt. *Journal of Mycopathological Research*, 43(1), 137-138.
- Chitwood-Brown, J., Vallad, G. E., Lee, T. G., & Hutton, S. F. (2021). Breeding for resistance to *Fusarium* wilt of tomato: A review. *Genes*, 12(11), 1673.
- Coates, J. (2006). Interpretation of infrared spectra, a practical approach. In R.A. Meyers *Encyclopedia of Analytical Chemistry*.
- Crestini, C., Lange, H., Sette, M., & Argyropoulos, D. S. (2017). On the structure of softwood kraft lignin. *Green Chemistry*, 19(17), 4104-4121.
- Cubero, O. F., Crespo, A. N. A., Fatehi, J., & Bridge, P. D. (1999). DNA extraction and PCR

- amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution*, 216, 243-249.
- Cuellar-Rufino, S., Arroyo-Xochihua, O., Salazar-Luna, A., & Arroyo-Helguera, O. (2022). Iodine induces toxicity against *Candida albicans* and *Candida glabrata* through oxidative stress. *Iranian Journal of Microbiology*, 14(2), 260.
- Dai, D. Q., Phookamsak, R., Wijayawardene, N. N., Li, W. J., Bhat, D. J., Xu, J. C., Taylor, J. E., Hyde, K. D., & Chukeatirote, E. (2017). Bambusicolous fungi. *Fungal Diversity*, 82, 1-105.
- Dai, Z., Shi, X., Liu, H., Li, H., Han, Y., & Zhou, J. (2018). High-strength lignin-based carbon fibers via a low-energy method. *RSC Advances*, 8(3), 1218–1224.
- Darabant, A., Rai, P. B., Staudhammer, C. L., & Dorji, T. (2016). Designing and evaluating bamboo harvesting methods for local needs: Integrating local ecological knowledge and science. *Environmental Management*, 58(2), 312-322.
- Dattilo, S., Spitaleri, F., Aleo, D., Saita, M. G., & Patti, A. (2023). Solid-state preparation and characterization of 2-hydroxypropylcyclodextrins-iodine complexes as stable iodophors. *Biomolecules*, 13(3), 474.
- Dessalegn, Y., Singh, B., van Vuure, A. W., Rajhi, A. A., Ahmed, G. M. S., & Hossain, N. (2022). Influence of age and harvesting season on the tensile strength of bamboo-fibre-reinforced epoxy composites. *Materials*, 15(12), 1–16.
- Domínguez-Robles, J., Tamminen, T., Liitiä, T., Peresin, M. S., Rodríguez, A., & Jääskeläinen, A. S. (2018). Aqueous acetone fractionation of kraft, organosolv and soda lignins. *International Journal of Biological Macromolecules*, 106, 979-987.
- Dong, Y., Liu, X., Liu, J., Yan, Y., Liu, X., Wang, K., & Li, J. (2021). Evaluation of anti-mold, termite resistance and physical-mechanical properties of bamboo cross-linking

- modified by polycarboxylic acids. *Construction and Building Materials*, 272, 121953.
- Duan, C., Meng, M., Huang, H., Wang, H., Zhang, Q., Gan, W., Ding, H., Zhang, J., Tang, X., & Pan, C. (2023). Performance and characterization of bamboo-based activated carbon prepared by boric acid activation. *Materials Chemistry and Physics*, 295, 127130.
- El-Nagar, A., Elzaawely, A. A., Xuan, T. D., Gaber, M., El-Wakeil, N., El-Sayed, Y., & Nehela, Y. (2022). Metal complexation of bis-chalcone derivatives enhances their efficacy against *Fusarium* wilt disease, caused by *Fusarium equiseti*, via Induction of Antioxidant Defense Machinery. *Plants*, 11(18), 2418.
- El-Samawaty, A. E. R. M. A., El-Wakil, D. A., Alamery, S., & Mahmoud, M. M. H. (2021). Potency of plant extracts against *Penicillium* species isolated from different seeds and fruits in Saudi Arabia. *Saudi Journal of Biological Sciences*, 28(6), 3294–3302.
- Emamverdian, A., Ding, Y., Ranaei, F., & Ahmad, Z. (2020). Application of Bamboo Plants in Nine Aspects. *Scientific World Journal*, 2020, 1-9.
- Fahim, A., Tan, Q., Bhatti, U. A., Nawaz, S. A., & Kaleri, A. H. (2022). Urban diversity impact on plant species due to environmental conditions. *Polish Journal of Environmental Studies*, 31(2), 1617-1623.
- Fanjul-Bolado, P., Fogel, R., Limson, J., Purcarea, C., & Vasilescu, A. (2020). Advances in the detection of dithiocarbamate fungicides: Opportunities for biosensors. *Biosensors*, 11(1), 12.
- Felisberto, M. H. F., Beraldo, A. L., Sentone, D. T., Klosterhoff, R. R., Clerici, M. T. P. S., & Cordeiro, L. M. C. (2021). Young culm of *Dendrocalamus asper*, *Bambusa tuldoidea* and *B. vulgaris* as sources of hemicellulosic dietary fibers for the food industry. *Food Research International*, 140(1), 109866.

- Ferreira, P. F., Pereira, A. L., Rosa, M. F., & de Santiago-Aguiar, R. S. (2022). Lignin-rich cellulose nanocrystals from coir fiber treated with ionic liquids: Preparation and evaluation as pickering emulsifier. *Industrial Crops and Products*, 186(1), 115119.
- Garajová, S., Patel, I., Lomascolo, A., Legée, F., Cézard, L., Cottyn, B., Lecourt, m., Bertrand, E., Sciara, G., Tapin-Lingua, S., Baumberger., Faulds, C. B., & Record, E. (2021). Treatment of wood fibres with laccases: Improved hardboard properties through phenolic oligomerization. *European Journal of Wood and Wood Products*, 79, 1369-1382.
- Ginoble Pandoli, O., Martins, R. S., De Toni, K. L. G., Paciornik, S., Maurício, M. H. P., Lima, R. M. C., Padilha, N. B., Letichevsky, S., Avillez, R. R., & Rodrigues, E. J. R. (2019). A regioselective coating onto microarray channels of bamboo with chitosan-based silver nanoparticles. *Journal of Coatings Technology and Research*, 16(1), 999-1011.
- Gmur, M. K., & Karpiński, T. M. (2020). Povidone-iodine in wound healing and prevention of wound infections. *European Journal of Biological Research*, 10(3), 232-239.
- Golubkina, N., Gomez, L. D., Kekina, H., Cozzolino, E., Simister, R., Tallarita, A., Valentina, T., Koshevarov, A., Cuciniello, A., Maiello, R., Cenvinzo, V., & Caruso, G. (2020). Joint selenium-iodine supply and Arbuscular Mycorrhizal fungi inoculation affect yield and quality of chickpea seeds and residual biomass. *Plants*, 9(7), 804.
- Gordobil, O., Herrera, R., Yahyaoui, M., İlk, S., Kaya, M., & Labidi, J. (2018). Potential use of kraft and organosolv lignins as a natural additive for healthcare products. *RSC Advances*, 8(43), 24525-24533.
- Govindaraj, C., & Robinson, P. (2020). Production and characterization of extracellular compounds from *Pseudomonas aeruginosa* and their impact on *Dendrocalamus asper*

- under in vitro stress condition. *Journal of Pharmacognosy and Phytochemistry*, 9(3), 726-734.
- Grozema, F. C., Zijlstra, R. W. J., Swart, M., & Duijnen, P. T. H. V. A. N. (1999). Iodine-benzene charge-transfer complex: Potential energy surface and transition probabilities studied at several levels of theory. *International Journal of Quantum Chemistry*, 75(4-5), 709-723.
- Guleria, V., Choudhary, P., Vashisth, A., Gupta, A., & Selvan, T. (2020). Studies on the physical characteristics, properties and productivity potential of exotic and indigenous bamboo in rainfed subtropical environment. *Journal of Pharmacognosy and Phytochemistry*, 9(3), 1552-1558.
- Hallur, V., Prakash, H., Sable, M., Preetam, C., Purushotham, P., Senapati, R., Shankarnarayan, S. A., Bag, N. D., & Rudramurthy, S. M. (2021). *Cunninghamella arunalokei* a new species of *Cunninghamella* from India causing disease in an immunocompetent individual. *Journal of Fungi*, 7(8), 670.
- Hami, A., Rasool, R. S., Khan, N. A., Mansoor, S., Mir, M. A., Ahmed, N., & Masoodi, K. Z. (2021). Morpho-molecular identification and first report of *Fusarium equiseti* in causing chili wilt from Kashmir (Northern Himalayas). *Scientific Reports*, 11(1), 3610.
- Han, L., Jiang, M., Zhang, J., Shao, C., & Zhang, Q. (2022). Thermal degradation and product analysis of 3-iodo-2-propyl-butylcarbamate as a wood preservative. *Polymers*, 14(21), 4531.
- Hartono, R., Iswanto, A. H., Priadi, T., Herawati, E., Farizky, F., Sutiawan, J., & Sumardi, I. (2022). Physical, chemical, and mechanical properties of six bamboo from Sumatera Island Indonesia and its potential applications for composite materials. *Polymers*, 14(22), 4868.

- Hassan, M. Z., Roslan, S. A., Sapuan, S. M., Rasid, Z. A., Mohd Nor, A. F., Md Daud, M. Y., Dolah, R., & Mohamed Yusoff, M. Z. (2020). Mercerization optimization of bamboo (*bambusa vulgaris*) fiber-reinforced epoxy composite structures using a box-behnken design. *Polymers*, 12(6), 1367.
- Hatch-McChesney, A., & Lieberman, H. R. (2022). Iodine and iodine deficiency: A comprehensive review of a re-emerging issues. *Nutrients*, 14(17), 3474.
- Hilgers, R., Vincken, J. P., Gruppen, H., & Kabel, M. A. (2018). Laccase/mediator systems: their reactivity toward phenolic lignin structures. *ACS Sustainable Chemistry & Engineering*, 6(2), 2037-2046.
- Hou, Y., Chen, J., Pan, D., & Zhao, L. (2023). Directional-freezing-assisted in situ sol-gel strategy to synthesize high-strength, fire-resistant, and hydrophobic wood-based composite aerogels for thermal insulation. *Gels*, 9(2), 17.
- Houbraken, J. A. M. P., & Samson, R. (2011). Phylogeny of *Penicillium* and the segregation of *Trichocomaceae* into three families. *Studies in Mycology*, 70(1), 1-51.
- Hwang, J. H., Jeong, H., Jung, Y. O., Nam, K. T., & Lim, K. M. (2021). Skin irritation and inhalation toxicity of biocides evaluated with reconstructed human epidermis and airway models. *Food and Chemical Toxicology*, 150, 112064.
- Ihssen, J., Schubert, M., Thöny-Meyer, L., & Richter, M. (2014). Laccase catalyzed synthesis of iodinated phenolic compounds with antifungal activity. *PLoS ONE*, 9(3), 1-12.
- Jaiswal, A. K., Alkan, N., Elad, Y., Sela, N., Graber, E. R., & Frenkel, O. (2020). Molecular insights into biochar-mediated plant growth promotion and systemic resistance in tomato against *Fusarium* crown and root rot disease. *Scientific reports*, 10(1), 13934.
- Jiang, N., Li, J., & Tian, C. M. (2018). *Arthrinium* species associated with bamboo and reed

- plants in China. *Fungal Systematics and Evolution*, 2(1), 1-9.
- Jiang, B., Zhang, Y., Zhao, H., Guo, T., Wu, W., & Jin, Y. (2019). Structure-antioxidant activity relationship of active oxygen catalytic lignin and lignin-carbohydrate complex. *International Journal of Biological Macromolecules*, 139, 21-29.
- Jiang, X., Wang, W., Guo, Y., & Dai, M. (2023). Effect of pressurized hydrothermal treatment on the properties of cellulose amorphous region based on molecular dynamics simulation. *Forests*, 14(2), 314.
- Jose, L. M., Kuriakose, S., & Thomas, S. (2020). Fabrication, characterization and in vitro antifungal property evaluation of biocompatible lignin-stabilized zinc oxide nanoparticles against selected pathogenic fungal strains. *BioNanoScience*, 10, 583-596.
- Kar, S., & Sutar, P. P. (2022). Enhancing the efficacy of microwave blanching-cum-black mould inactivation of whole garlic (*Allium sativum* L.) bulbs using ultrasound: Higher inactivation of peroxidase, polyphenol oxidase, and *Aspergillus niger* at lower processing temperatures. *Food and Bioprocess Technology*, 15(3), 635-655.
- Karlinasari, L., Sejati, P. S., Adzkie, U., Arinana, A., & Hiziroglu, S. (2021). Some of the physical and mechanical properties of particleboard made from betung bamboo (*Dendrocalamus asper*). *Applied Sciences*, 11(8), 3682.
- Kato, T., Hatakeyama, T., & Sugawara, K. (2021). Release behavior of arsenic, chromium, and copper during heat treatments of CCA-treated wood. *Journal of Material Cycles and Waste Management*, 23(4), 1636-1645.
- Kawai, S., Asukai, M., Ohya, N., Okita, K., Ito, T., & Ohashi, H. (1999). Degradation of a non-phenolic β -O-4 substructure and of polymeric lignin model compounds by laccase of *Coriolus versicolor* in the presence of 1-hydroxybenzotriazole. *FEMS Microbiology Letters*, 170(1), 51-57.

- Kenned, J. J., Sankaranarayananasamy, K., & Kumar, C. S. (2021). Chemical, biological, and nanoclay treatments for natural plant fiber-reinforced polymer composites: A review. *Polymers and Polymer Composites*, 29(7), 1011-1038.
- Kilpi-Koski, J., Penttinen, O. P., Väisänen, A. O., & van Gestel, C. A. (2020). Toxicity of binary mixtures of Cu, Cr and As to the earthworm *Eisenia andrei*. *Ecotoxicology*, 29, 900-911.
- Kolasiński, W. (2019). Surgical site infections - review of current knowledge, methods of prevention. *Polish Journal of Surgery*, 91(4), 41-47.
- Kong, C. K., Tan, Y. N., Chye, F. Y., & Sit, N. W. (2020). Nutritional compositions, biological activities, and phytochemical contents of the edible bamboo shoot, *Dendrocalamus asper*, from Malaysia. *International Food Research Journal*, 27(3), 54-54.
- Kumar, S., Rawat, D., Singh, B., & Khanduri, V. P. (2023). Utilization of bamboo resources and their market value in the western Himalayan region of India. *Advances in Bamboo Science*, 3(1), 100019.
- Laut, S., Poapolathep, S., Piasai, O., Sommai, S., Boonyuen, N., Giorgi, M., Zhang, Z., Fink-Grimmels, J., & Poapolathep, A. (2023). Storage fungi and mycotoxins associated with rice samples commercialized in Thailand. *Foods*, 12(3), 487.
- Lee, J. H., Kim, K., Jin, X., Kim, T. M., Choi, I. G., & Choi, J. W. (2021). Formation of pure nanoparticles with solvent-fractionated lignin polymers and evaluation of their biocompatibility. *International Journal of Biological Macromolecules*, 183, 660-667.
- Leskinen, T., Smyth, M., Xiao, Y., Lintinen, K., Mattinen, M. L., Kostiaainen, M. A., Oinas, P., & Österberg, M. (2017). Scaling up production of colloidal lignin particles. *Nordic Pulp & Paper Research Journal*, 32(4), 586-596.

- Li, D., Xu, C., Yeager, C. M., Lin, P., Xing, W., Schwehr, K. A., Chen, N., Arthur, Z., Kaplan, D. I., & Santschi, P. H. (2019). Molecular interaction of aqueous iodine species with humic acid studied by I and C K-edge x-ray absorption spectroscopy. *Environmental Science & Technology*, 53(21), 12416–12424.
- Li, F., Huang, X., Liu, J. X., & Zhang, G. J. (2020). Sol-gel derived porous ultra-high temperature ceramics. *Journal of Advanced Ceramics*, 9, 1-16.
- Li, J., Ma, R., Wu, Z., He, S., Chen, Y., Bai, R., & Wang, J. (2021). Visible-light-driven Ag-modified TiO₂ thin films anchored on bamboo material with antifungal memory activity against *Aspergillus niger*. *Journal of Fungi*, 7(8), 592.
- Li, N., Noro, J., Su, J., Wang, H., Silva, C., & Cavaco-Paulo, A. (2022). Enhancing laccase-assisted polymerization reactions with perfluorinated compounds. *Biochemical Engineering Journal*, 189, 108736.
- Liao, M., Ren, X., Gao, Q., Liu, N., Tang, F., Wang, G., & Cao, H. (2021). Anti-fungal activity of moso bamboo (*Phyllostachys pubescens*) leaf extract and its development into a botanical fungicide to control pepper phytophthora blight. *Scientific Reports*, 11(1), 1–10.
- Lievonen, M., Valle-Delgado, J. J., Mattinen, M. L., Hult, E. L., Lintinen, K., Kostinen, M. A., Paananen, A., Szilvay, G. R., Setälä, H., & Österberg, M. (2016). A simple process for lignin nanoparticle preparation. *Green Chemistry*, 18(5), 1416–1422.
- Lin, T., Li, L., Gu, X., Owusu, A. M., Li, S., Han, S., Cao, G., Zhu, T., & Li, S. (2023). Seasonal variations in the composition and diversity of rhizosphere soil microbiome of bamboo plants as infected by soil-borne pathogen and screening of associated antagonistic strains. *Industrial Crops and Products*, 197, 116641.
- Liu, J., Li, X., & Lu, J. (2018). First report of blue mold on *Sparassis crispa* caused by

- Penicillium sumatrense* in China. *Plant disease*, 102(11), 2380-2380.
- Long, J., Qian, Y., Tian, W., Li, N., Chen, D., Xu, Q., Li, H., & Lu, J. (2023). Enhanced piezocatalytic activity of BiFeO₃ hollow spheres with surface iodine-grafting. *Chemical Engineering Science*, 278, 118936.
- Lotfy, V. F., & Basta, A. H. (2022). A green approach to the valorization of kraft lignin for the production of nanocomposite gels to control the release of fertilizer. *Biofuels, Bioproducts and Biorefining*, 16(2), 488-498.
- Lozovaya, V. V., Lygin, A. V., Zernova, O. V., Li, S., Widholm, J. M., & Hartman, G. L. (2006). Lignin degradation by *Fusarium solani* f. sp. *glycines*. *Plant Disease*, 90(1), 77-82.
- Lu, B. H., Zuo, B., Liu, X. L., Feng, J., Wang, Z. M., & Gao, J. (2016). *Trichoderma harzianum* causing green mold disease on cultivated *Ganoderma lucidum* in Jilin Province, China. *Plant Disease*, 100(12), 2524.
- Lu, Y., Qiu, J., Wang, S., Xu, J., Ma, G., Shi, J., & Bao, Z. (2021). Species diversity and toxigenic potential of *Fusarium incarnatum-equiseti* species complex isolates from rice and soybean in China. *Plant Disease*, 105(9), 2628-2636.
- Luciano-Rosario, D., Keller, N. P., & Jurick, W. M. (2020). *Penicillium expansum*: Biology, omics, and management tools for a global postharvest pathogen causing blue mould of pome fruit. *Molecular Plant Pathology*, 21(11), 1391-1404.
- Luo, T., Wang, C., Ji, X., Yang, G., Chen, J., Janaswamy, S., & Lyu, G. (2021). Preparation and characterization of size-controlled lignin nanoparticles with deep eutectic solvents by nanoprecipitation. *Molecules*, 26(1), 218.
- Lv, B. W., Xu, H., Guo, J. Z., Bai, L. Q., & Li, B. (2022). Efficient adsorption of methylene blue on carboxylate-rich hydrochar prepared by one-step hydrothermal carbonization

- of bamboo and acrylic acid with ammonium persulphate. *Journal of Hazardous Materials*, 421, 126741.
- Ma, R., Chen, Y., Yang, Y., Wu, Z., Bao, Y., Li, N., & Li, J. (2021). Facile hydrothermal deposition of octahedral-shaped Cu₂O crystallites on bamboo veneer for efficient degradation of organic aqueous solution. *Vacuum*, 185, 110038.
- Ma, Y., Liao, Y., Jiang, Z., Sun, Q., Guo, X., Zhang, W., Hu, C., Luque, R., Shi, B., & Sels, B. F. (2023). Solvent effect on the production of spherical lignin nanoparticles. *Green Chemistry*, 25(3), 993-1003.
- Mahdian, S., & Zafari, D. (2017). First report of table grape blue mold caused by *Penicillium sumatrense* in Iran. *Plant Disease*, 101(1), 244-244.
- Makhayeva, D. N., Filippov, S. K., Yestemes, S. S., Irmukhametova, G. S., Khutoryanskiy, V. V. (2022). Polymeric iodophors with poly (2-ethyl-2-oxazoline) and poly (N-vinylpyrrolidone): Optical, hydrodynamic, thermodynamic, and antimicrobial properties. *European Polymer Journal*, 165, 111005.
- Manghwar, H., Hussain, A., Ali, Q., Saleem, M. H., Abualreesh, M. H., Alatawi, A., Ali, S., & Munis, M. F. H. (2021). Disease severity, resistance analysis, and expression profiling of pathogenesis-related protein genes after the inoculation of *Fusarium equiseti* in wheat. *Agronomy*, 11(11), 2124.
- Manisekaran, A., Grysan, P., Duez, B., Schmidt, D. F., Lenoble, D., & Thomann, J. S. (2022). Solvents drive self-assembly mechanisms and inherent properties of Kraft lignin nanoparticles (<50 nm). *Journal of Colloid and Interface Science*, 626, 178-192.
- Martin, E., Badel, E., Léger, S., Dubessay, P., Delattre, C., Audonnet, F., Hartmann, F., Bertrand, E., Sciara, G., Garajova, S., Record, E., de Baynast, H., & Michaud, P. (2022). Effect of laccase pre-treatment on the mechanical properties of lignin-based

- agrocomposites reinforced with wood fibers. *Industrial Crops and Products*, 189, 115876.
- Masisi, B., Zabel, A., Blaser, J., & Augustino, S. (2022). Fighting climate change with bamboo in Africa: The case of Kyela, Rungwe and Mufindi districts–Tanzania. *Advances in Bamboo Science*, 1, 100009.
- Matsakas, L., Gerber, M., Yu, L., Rova, U., & Christakopoulos, P. (2020). Preparation of low carbon impact lignin nanoparticles with controllable size by using different strategies for particles recovery. *Industrial Crops and Products*, 147, 112243.
- McDonnell, G., & Russell, A. D. (1999). Antiseptics and disinfectants: activity, action, and resistance. *Clinical Microbiology Reviews*, 12(1), 147-179.
- Melro, E., Filipe, A., Sousa, D., Medronho, B., & Romano, A. (2021). Revisiting lignin: A tour through its structural features, characterization methods and applications. *New Journal of Chemistry*, 45(16), 6986-7013.
- Mita, A., Hirano, S., Uehara, T., Uehara, K., Ohno, Y., Kubota, K., & Masuda, Y. (2022). Fatal disseminated mucormycosis due to *Cunninghamella bertholletiae* infection after ABO - incompatible living donor liver transplantation: A case report. *Surgical Case Reports*, 8(1), 1-7.
- Mo, D., , X., Chen, Y., Jiang, Y., Gan, C., Zhang, Y., Li, W., Huang, Y., & Cui, J. (2021). Fabrication and evaluation of slow-release lignin-based avermectin nano-delivery system with UV-shielding property. *Scientific Reports*, 11(1), 23248.
- Mohan, N., Dash, S. P., Bobby, N. M., & Shetty, D. (2022). Study of bamboo as a building material- Construction & preservation techniques and its sustainability. *Materials Today: Proceedings*, 60, 100-114.
- Mondal, D., Roy, K., Pal, D., & Koley, D. K. (2022). Deep learning-based approach to detect

- and classify signs of crop leaf diseases and pest damage. *SN Computer Science*, 3(6), 433.
- Morais, S., Fonseca, H. M., Oliveira, S. M., Oliveira, H., Gupta, V. K., Sharma, B., & de Lourdes Pereira, M. (2021). Environmental and health hazards of chromated copper arsenate-treated wood: A review. *International journal of environmental research and public health*, 18(11), 5518.
- Morales, A., Labidi, J., & Gullón, P. (2022). Impact of the lignin type and source on the characteristics of physical lignin hydrogels. *Sustainable Materials and Technologies*, 31, 369.
- Morena, A. G., Bassegoda, A., Natan, M., Jacobi, G., Banin, E., & Tzanov, T. (2022). Antibacterial properties and mechanisms of action of sonoenzymatically synthesized lignin-based nanoparticles. *ACS Applied Materials & Interfaces*, 14(33), 37270-37279.
- Mustafa, A. A., Derise, M. R., Yong, W. T. L., & Rodrigues, K. F. (2021). A concise review of *Dendrocalamus asper* and related bamboos: Germplasm conservation, propagation and molecular biology. *Plants*, 10(9), 1897.
- Nakhaei, M., Akhbari, K., & Davoodi, A. (2021). Biocompatible MOF-808 as an iodophor antimicrobial agent with controlled and sustained release of iodine. *Crystal Engineering and Communication*, 23(48), 8538-8545.
- Nair, S. S., Sharma, S., Pu, Y., Sun, Q., Pan, S., Zhu, J. Y., Deng, Y., & Ragauskas, A. J. (2014). High shear homogenization of lignin to nanolignin and thermal stability of nanolignin-polyvinyl alcohol blends. *ChemSusChem*, 7(12), 3513-3520.
- Navarro, M. V., de Barros, Y. N., Segura, W. D., Chaves, A. F. A., Jannuzzi, G. P., Ferreira, K. S., Xander, P., & Batista, W. L. (2021). The role of dimorphism regulating histidine kinase (Drk1) in the pathogenic fungus *Paracoccidioides brasiliensis* cell wall. *Journal*

of Fungi, 7(12), 1014.

Nefzi, A., Abdallah, R. A. B., Jabnoun-Khiareddine, H., Ammar, N., & Daami-Remadi, M.

(2019). Ability of endophytic fungi associated with *Withania somnifera* L. to control *Fusarium* Crown and Root Rot and to promote growth in tomato. *Brazilian Journal of Microbiology*, 50, 481-494.

Nelson, J., Pickering, K. L., & Beg, M. D. (2023). Assessment of the potential of waste copper chromium and arsenic (CCA)-treated timber fibre reinforced polypropylene composites for construction. *Journal of Composites Science*, 7(2), 48.

Nikolaivits, E., Siaperas, R., Agrafiotis, A., Ouazzani, J., Magoulas, A., Gioti, A., & Topakas, E. (2021). Functional and transcriptomic investigation of laccase activity in the presence of PCB29 identifies two novel enzymes and the multicopper oxidase repertoire of a marine-derived fungus. *Science of the Total Environment*, 775, 145818.

O'Brien, D. M., Vallieres, C., Alexander, C., Howdle, S. M., Stockman, R. A., & Avery, S. V. (2019). Epoxy-amine oligomers from terpenes with applications in synergistic antifungal treatments. *Journal of Materials Chemistry B*, 7(34), 5222-5229.

Österberg, M., Sipponen, M. H., Mattos, B. D., & Rojas, O. J. (2020). Spherical lignin particles: A review on their sustainability and applications. *Green Chemistry*, 22(9), 2712-2733.

Otero, C., Arredondo, C., Echeverría-Vega, A., & Gordillo-Fuenzalida, F. (2020). *Penicillium* spp. mycotoxins found in food and feed and their health effects. *World Mycotoxin Journal*, 13(3), 323-343.

Panda, L., & Duarte-Sierra, A. (2022). Recent advancements in enhancing antimicrobial activity of plant-derived polyphenols by biochemical means. *Horticulturae*, 8(5), 401.

Papadopoulos, A. N., & Taghiyari, H. R. (2019). Innovative wood surface treatments based

- on nanotechnology. *Coatings*, 9(12), 866.
- Patriarca, A. (2019). Fungi and mycotoxin problems in the apple industry. *Current Opinion in Food Science*, 29(1), 42-47.
- Pehrsson, P. R., Roseland, J. M., Patterson, K. Y., Phillips, K. M., Spungen, J. H., Andrews, K. W., Gusev, P. A., Gahche, J. J., Haggans, C. J., & Merkel, J. M. (2022). Iodine in foods and dietary supplements: A collaborative database developed by NIH, FDA and USDA. *Journal of Food Composition and Analysis*, 109, 104369.
- Pérez-Pazos, J., Rosero, A., Cardinale, M., & Gámez, R. (2023). Development of control strategies for bacteria and fungi associated with a micropropagated new cultivar of orange-fleshed sweet potato (*Ipomoea batatas* cv. Agrosavia–Aurora). *Horticulture, Environment, and Biotechnology*, 1, 1-17.
- Perincherry, L., Lalak-Kańczugowska, J., & Stępień, Ł. (2019). *Fusarium*-produced mycotoxins in plant-pathogen interactions. *Toxins*, 11(11), 664.
- Pfordt, A., Schiwek, S., Karlovsky, P., & von Tiedemann, A. (2020). *Trichoderma afroharzianum* ear rot—a new disease on maize in Europe. *Frontiers in Agronomy*, 2(1) 11.
- Pham, C. V., & Tran, H. T. (2021). *Cunninghamella elegans* causing cassava root rot in Vietnam. *Australasian Plant Disease Notes*, 16(1), 14.
- Phookamsak, R., Jiang, H., Suwannarach, N., Lumyong, S., Xu, J., Xu, S., Liao, C.-F., & Chomnunti, P. (2022). Bambusicolous fungi in *Pleosporales*: Introducing four novel taxa and a new habitat record for *Anastomitrabeculia didymospora*. *Journal of Fungi*, 8(6), 630.
- Prosper, N. K., Zhang, S., Wu, H., Yang, S., Li, S., Sun, F., & Goodell, B. (2018). Enzymatic biocatalysis of bamboo chemical constituents to impart antimold properties. *Wood*

- Science and Technology*, 52(3), 619-635.
- Qu, L., Rahimi, S., Qian, J., He, L., He, Z., & Yi, S. (2021). Preparation and characterization of hydrophobic coatings on wood surfaces by a sol-gel method and post-aging heat treatment. *Polymer Degradation and Stability*, 183, 109429.
- Raimond, L., Halbus, A. F., Athab, Z. H., & Paunov, V. N. (2022). Antimould action of Ziram and IPBC loaded in functionalised nanogels against *Aspergillus niger* and *Penicillium chrysogenum*. *Materials Advances*, 3(22), 8178-8192.
- Ralph, J., Lapierre, C., & Boerjan, W. (2019). Lignin structure and its engineering. *Current Opinion in Biotechnology*, 56, 240-249.
- Ranjan, J., Joshi, V., Mandal, T., & Mandal, D. D. (2021). Ecotoxicological risk assessment of pentachlorophenol, an emerging DBP to plants: evaluation of oxidative stress and antioxidant responses. *Environmental Science and Pollution Research*, 28, 27954-27965.
- Ravi, M., Rao, G. P., Meshram, N. M., & Sundararaj, R. (2022). Genetic diversity of phytoplasmas associated with several bamboo species in India. *Forest Pathology*, 52(3), e12741.
- Ren, D., Li, J., Bao, Y., Wu, Z., He, S., Wang, A., Guo, F., Chen, Y., & Chen, Y. (2018). Low-temperature synthesis of flower-like ZnO microstructures supported on TiO₂ thin films as efficient antifungal coatings for bamboo protection under dark conditions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 555, 381-388.
- Rich, J. O., Anderson, A. M., & Berhow, M. A. (2016). Laccase-mediator catalyzed conversion of model lignin compounds. *Biocatalysis and Agricultural Biotechnology*, 5, 111-115.
- Richter, A. P., Bharti, B., Armstrong, H. B., Brown, J. S., Plemmons, D., Paunov, V. N.,

- Stoyanov, S. D., & Velez, O. D. (2016). Synthesis and characterization of biodegradable lignin nanoparticles with tunable surface properties. *Langmuir*, 32(25), 6468-6477.
- Ringman, R., Pilgård, A., Brischke, C., & Richter, K. (2014). Mode of action of brown rot decay resistance in modified wood: A review. *Holzforschung*, 68(2), 239-246.
- Riyazuddin, R., Singh, K., Iqbal, N., Nisha, N., Rani, A., Kumar, M., Khatri, N., Siddiqui, M.H., Yaheshwar, Kim, S.T., & Gupta, R. (2023). Iodine: An emerging biostimulant of growth and stress responses in plants. *Plant and Soil*, 486(1-2), 119-133.
- Rudramurthy, S. M., Paul, R. A., Chakrabarti, A., Mouton, J. W., & Meis, J. F. (2019). Invasive aspergillosis by *Aspergillus flavus*: epidemiology, diagnosis, antifungal resistance, and management. *Journal of Fungi*, 5(3), 55.
- Schneider, W. D. H., Dillon, A. J. P., & Camassola, M. (2021). Lignin nanoparticles enter the scene: A promising versatile green tool for multiple applications. *Biotechnology Advances*, 47, 107685.
- Scussel, R., Feltrin, A. C., Angioletto, E., Galvani, N. C., Fagundes, M. Í., Bernardin, A. M., Feuser, P. E., de Ávila, R. A. M., & Pich, C. T. (2022). Ecotoxic, genotoxic, and cytotoxic potential of leachate obtained from chromated copper arsenate-treated wood ashes. *Environmental Science and Pollution Research*, 29(27), 41247-41260.
- Sdahl, M., Conrad, J., Braunberger, C., & Beifuss, U. (2019). Efficient and sustainable laccase-catalyzed iodination of P-substituted phenols using KI as iodine source and aerial O₂ as oxidant. *RSC Advances*, 9(34), 19549-19559.
- Sebaa, N. A., Zatl, A. T., Dib, M. E., Tabti, B., Costa, J., & Muselli, A. (2019). Antifungal activity of essential oil and hydrosol extract of *Ballota nigra* L. and their protective effects against the black rot of tomatoes. *Current Nutrition & Food Science*, 15(7), 662-

- Segura-Palacios, M. A., Correa-Pacheco, Z. N., Corona-Rangel, M. L., Martinez-Ramirez, O. C., Salazar-Piña, D. A., Ramos-García, M. de L., & Bautista-Baños, S. (2021). Use of natural products on the control of *Aspergillus flavus* and production of aflatoxins in vitro and on tomato fruit. *Plants*, 10(12), 1-9.
- Sethy, P. K., Barpanda, N. K., Rath, A. K., & Behera, S. K. (2020). Image processing techniques for diagnosing rice plant disease: A survey. *Procedia Computer Science*, 167, 516-530.
- Serris, A., Danion, F., & Lanternier, F. (2019). Disease entities in mucormycosis. *Journal of Fungi*, 5(1), 23.
- Shen, X. Y., Cheng, Y. L., Cai, C. J., Fan, L., Gao, J., & Hou, C. L. (2014). Diversity and antimicrobial activity of culturable endophytic fungi isolated from moso bamboo seeds. *PloS One*, 9(4), e95838.
- Shu, F., Jiang, B., Yuan, Y., Li, M., Wu, W., Jin, Y., & Xiao, H. (2021). Biological activities and emerging roles of lignin and lignin-based products- A review. *Biomacromolecules*, 22(12), 4905-4918.
- Sidahmed, M. S., & Nimir, A. H. H. (2020). Effect of peptone on the antifungal activity of povidone iodine. *International Journal of Current Microbiology and Applied Sciences*, 9(12), 1798–1802.
- Silva, M. F., Menis-Henrique, M. E., Felisberto, M. H., Goldbeck, R., & Clerici, M. T. (2020). Bamboo as an eco-friendly material for food and biotechnology industries. *Current Opinion in Food Science*, 33, 124-130.
- Singh, L., Sridharan, S., Thul, S. T., Kokate, P., Kumar, P., Kumar, S., & Kumar, R. (2020). Eco-rejuvenation of degraded land by microbe assisted bamboo plantation. *Industrial*

Crops and Products, 155, 112795.

- Singh, A., Kuzhikkali, V., & Haq, M. (2021). Irritant contact dermatitis with topical povidone-iodine ointment post ear surgery. *Ear, Nose & Throat Journal*, 100(3), 139-140.
- Soliman, S. S., Baldin, C., Gu, Y., Singh, S., Gebremariam, T., Swidergall, M., Alqarihi, A., Youssef, E. G., Alkhazraji, S., Pikoulas, A., Perske, C., Venkataramani, V., Rich, A., Bruno, V. M., Hotopp, J. D., mantis, N. J., Edward, J. E., Filler, S. G., Chamilos, G., Vitetta, E. S., & Ibrahim, A. S. (2021). Mucoricin is a ricin-like toxin that is critical for the pathogenesis of mucormycosis. *Nature microbiology*, 6(3), 313-326.
- Subekti, N., & Yoshimura, T. (2020). Activity of bamboo Wulung's smoke *Gigantochloa atroviolace* againts subterranean termites and fungi attack. *Journal of Agricultural Science*, 42(3), 541-547.
- Sun, Z., & Mingming, W. (2019). Effects of sol-gel modification on the interfacial and mechanical properties of sisal fiber reinforced polypropylene composites. *Industrial Crops and Products*, 137, 89-97.
- Sun, B. D., Visagie, C. M., Chen, A. J., & Houbraken, J. (2021). A taxonomic review of *Penicillium* section *Charlesia*. *Mycological Progress*, 20(11), 1383-1397.
- Tafa, T. G., & Engida, A. M. (2022). Preparation of green film with improved physicochemical properties and enhanced antimicrobial activity using ingredients from cassava peel, bamboo leaf and rosemary leaf. *Heliyon*, 8(8), e10130.
- Tian, D., Guo, Y., Huang, M., Zhao, L., Deng, S., Deng, O., Zhou, W., Hu, J., & Shen, F (2021). Bacterial cellulose/lignin nanoparticles composite films with retarded biodegradability. *Carbohydrates Polymers*, 274, 118656.
- Tran, N. T., Ha, D., Pham, L. H., Vo, T. V., Nguyen, N. N., Tran, C. K., Nguyen, D. M.,

- Nguyen, T. T. T., Tran, T. T. V., Nguyen, P. L. M., & Hoang, D. (2023). Ag/SiO₂ nanoparticles stabilization with lignin derived from rice husk for antifungal and antibacterial activities. *International Journal of Biological Macromolecules*, 230, 123124.
- Trevisan, H., & Rezende, C. A. (2020). Pure, stable and highly antioxidant lignin nanoparticles from elephant grass. *Industrial Crops and Products*, 145, 112105.
- Ullah, I., Chen, Z., Xie, Y., Khan, S. S., Singh, S., Yu, C., & Cheng, G. (2022). Recent advances in biological activities of lignin and emerging biomedical applications: A short review. *International Journal of Biological Macromolecules*, 208, 819-832.
- Vanholme, R., De Meester, B., Ralph, J., & Boerjan, W. (2019). Lignin biosynthesis and its integration into metabolism. *Current Opinion in Biotechnology*, 56, 230-239.
- Vasylychenko, A., & Derevianko, S. (2021). Antifungal activity of a composition of selenium and iodine nanoparticles. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 69(4), 491–500.
- Villegas, C. A. M., & Zagury, G. J. (2023). Incorporating oral, inhalation and dermal bioaccessibility into human health risk characterization following exposure to chromated copper arsenate (CCA)-contaminated soils. *Ecotoxicology and Environmental Safety*, 249, 114446.
- Vogel, G. F., & Fey, R. (2019). *Azospirillum brasilense* interaction effects with captan and thiodicarb on the initial growth of corn plants. *Revista de Agricultura Neotropical*, 6(3), 53-59.
- Wang, Y., Chen, J., Wang, D., Ye, F., He, Y., Hu, Z., & Zhao, G. (2020). A systematic review on the composition, storage, processing of bamboo shoots: Focusing the nutritional and functional benefits. *Journal of Functional Foods*, 71, 104015.

- Wang, J., Wang, H., Ye, Z., Chizaram, E. P., Jiang, J., Liu, T., Sun, F., & Zhang, S. (2020). Mold resistance of bamboo after laccase-catalyzed attachment of thymol and proposed mechanism of attachment. *RSC Advances*, 10(13), 7764-7770.
- Wei, F., Ma, N., Haseeb, H. A., Gao, M., Liu, X., & Guo, W. (2022). Insights into structural and physicochemical properties of maize starch after *Fusarium verticillioides* infection. *Journal of Food Composition and Analysis*, 114, 104819.
- Weng, K. F., Shi, Y., Zheng, X., & Phillips, D. L. (2006). Resonance Raman investigation of the short-time photodissociation dynamics of the charge-transfer absorption of the I₂-benzene complex in benzene solution. *Journal of Physical Chemistry A*, 110(3), 851–860.
- Weng, C., Peng, X., & Han, Y. (2021). Depolymerization and conversion of lignin to value-added bioproducts by microbial and enzymatic catalysis. *Biotechnology for Biofuels*, 14(1), 1-22.
- Wijaya, C. J., Ismadji, S., & Gunawan, S. (2021). A review of lignocellulosic-derived nanoparticles for drug delivery applications: Lignin nanoparticles, xylan nanoparticles, and cellulose nanocrystals. *Molecules*, 26(3), 676.
- Wijesinghe, S. N., Wang, Y., Camporesi, E., Wanasinghe, D. N., Boonmee, S., & Hyde, K. D. (2020). A new genus of *Bambusicolaceae* (*Pleosporales*) on *Corylus avellana* (*Fagales*) from Italy. *Biodiversity Data Journal*, 8, e55957.
- Wójcik, P. (2023). Effects of preharvest sprays of iodine, selenium and calcium on apple biofortification and their quality and storability. *Plos one*, 18(3), e0282873.
- Wu, X., Fan, Z., Wang, J., Wang, H., Han, S., Zhang, Y., & Sun, F. L. (2022). Improving the anti-mould capacity of bamboo through sequential alkaline extraction and laccase-mediated thymol modification. *Construction and Building Materials*, 354, 129104.

- Yan, J., Niu, Y., Wu, C., Shi, Z., Zhao, P., Naik, N., Mai, X., & Yuan, B. (2021). Antifungal effect of seven essential oils on bamboo. *Advanced Composites and Hybrid Materials*, 4(3), 552–561.
- Yang, S., Luo, S., Huang, A., Luo, Y., Li, D., Wu, Y., & Ji, N. (2019). Mold resistance of bamboo treated with copper complexes-grafted silica gel and its microdistribution in treated bamboo. *Journal of Wood Science*, 65(1), 62.
- Yiamsawas, D., Beckers, S. J., Lu, H., Landfester, K., & Wurm, F. R. (2017). Morphology-controlled synthesis of lignin nanocarriers for drug delivery and carbon materials. *ACS Biomaterials Science & Engineering*, 3(10), 2375-2383.
- You, T., Li, X., Wang, R., Zhang, X., & Xu, F. (2019). Effects of synergistic fungal pretreatment on structure and thermal properties of lignin from corncob. *Bioresource Technology*, 272, 123-129.
- Yuan, Y., Xi, B., He, X. S., Tan, W., Zhang, H., Li, D., Yang, C., & Zhao, X. (2019). Polarity and molecular weight of compost-derived humic acids impact bio-dechlorination of pentachlorophenol. *Journal of Agricultural and Food Chemistry*, 67(17), 4726-4733.
- Yuan, B., Guo, M., Murugadoss, V., Song, G., & Guo, Z. (2021). Immobilization of graphitic carbon nitride on wood surface via chemical crosslinking method for UV resistance and self-cleaning. *Advanced Composites and Hybrid Materials*, 4, 286-293.
- Yuan, T., Yin, X., Huang, Y., Li, X., Wang, X., Chen, L., & Li, Y. (2022). Hydrothermal treatment of bamboo and its effect on nano-mechanic and anti-mildew property. *Journal of Cleaner Production*, 380, 135189.
- Yves, K. G., Chen, T., Aladejana, J. T., Wu, Z., & Xie, Y. (2020). Preparation, test, and analysis of a novel aluminosilicate-based antimildew agent applied on the microporous structure of wood. *ACS Omega*, 5(15), 8784-8793.

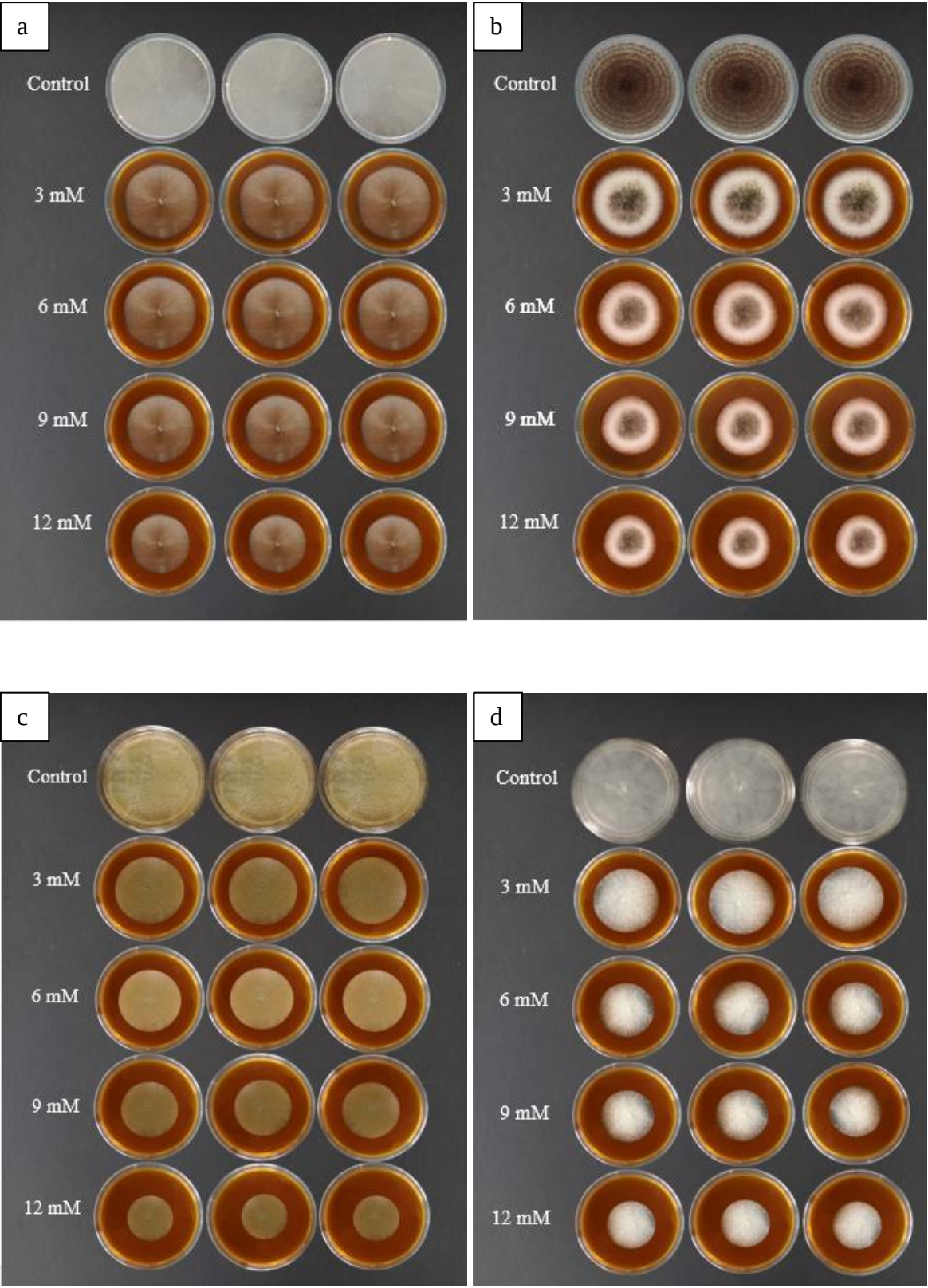
- Zerva, A., Koutroufini, E., Kostopoulou, I., Detsi, A., & Topakas, E. (2019). A novel thermophilic laccase-like multicopper oxidase from *Thermothelomyces thermophila* and its application in the oxidative cyclization of 2', 3, 4-trihydroxychalcone. *New Biotechnology*, 49, 10-18.
- Zhang, R., Li, Y., He, Y., & Qin, D. (2020). Preparation of iodopropynyl butyrcarbamate loaded halloysite and its anti-mildew activity. *Journal of Materials Research and Technology*, 9(5), 10148-10156.
- Zhang, J., Li, Y., Bao, Q., Wang, H., & Hou, S. (2022). Plant elicitor peptide 1 fortifies root cell walls and triggers a systemic root-to-shoot immune signaling in *Arabidopsis*. *Plant Signaling & Behavior*, 17(1), 2034270.
- Zhao, C., Huang, J., Yang, L., Yue, F., & Lu, F. (2019). Revealing structural differences between alkaline and kraft lignins by HSQC NMR. *Industrial & Engineering Chemistry Research*, 58(14), 5707-5714.
- Zhao, C., Hu, Z., Shi, L., Wang, C., Yue, F., Li, S., Zhang, H., & Lu, F. (2020). Profiling of the formation of lignin-derived monomers and dimers from Eucalyptus alkali lignin. *Green Chemistry*, 22(21), 7366-7375
- Zheng, Y., Guo, M., Zhou, Q., & Liu, H. (2019). Effect of lignin degradation product sinapyl alcohol on laccase catalysis during lignin degradation. *Industrial Crops and Products*, 139, 111544.
- Zikeli, F., Vinciguerra, V., D'Annibale, A., Capitani, D., Romagnoli, M., & Scarascia Mugnozza, G. (2019). Preparation of lignin nanoparticles from wood waste for wood surface treatment. *Nanomaterials*, 9(2), 281.

APPENDICES

Appendix A: Journal Publications

1. **Amat, M.,** Chin, S. F., Ngieng, N. S., Wasli, M. E., & Husaini, A. A. S. A. (2024). Mold resistance of bamboo after laccase-catalyzed fixation of lignin nanoparticles. *ChemistrySelect*, 9(1), e202303235.
2. **Amat, M.,** Chin, S.F, Wasli, M. E., Ngieng, N. S., & Husaini, A. A. S. A. (2023). Particle sizes-dependent antifungal activity of lignin nanoparticles. *Chemical Engineering Transactions*, 106, 1333-1338.
3. **Amat, M.,** Chin, S. F., Wasli, M. E., Ngieng, N. S., & Husaini, A. A. S. A. (2023). Eco-friendly synthesis of lignin-based nanoparticles as antifungal agent. *Materials Letters*, 340, 134166.

Appendix B: Antifungal Activity



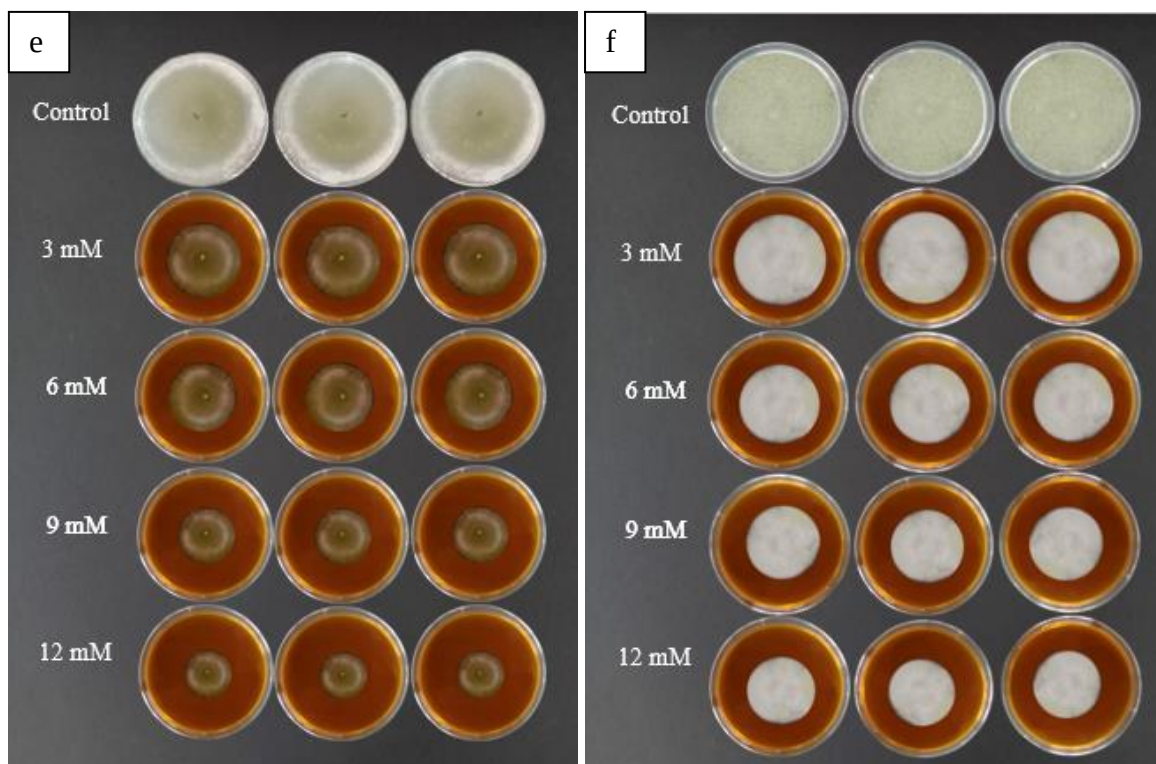
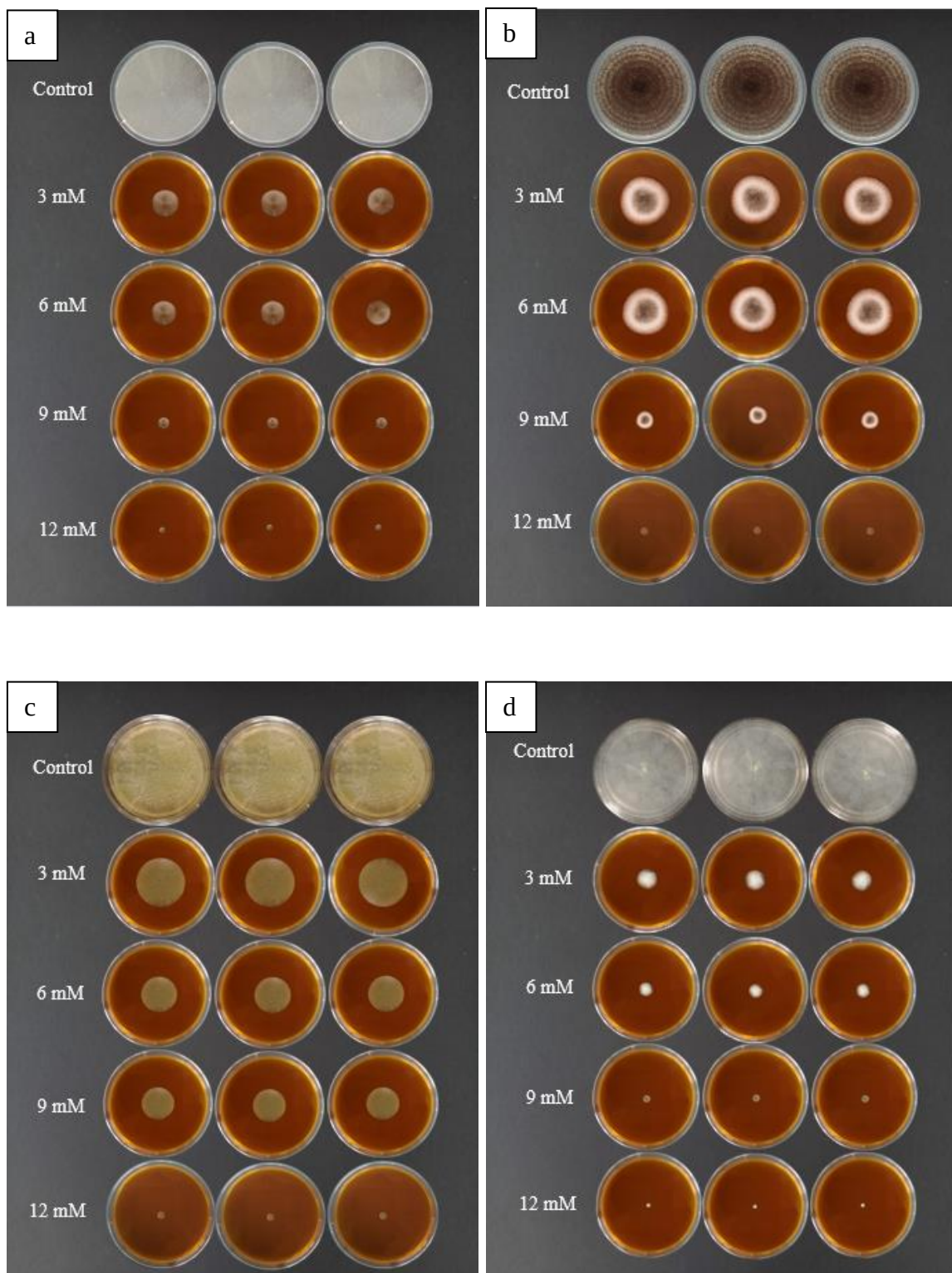


Figure 1: Macroscopic Image of Fungi Treated With LNPs at 20 nm of Various Concentrations Against (a) *F. equiseti* (b) *A. niger* (c) *A. flavus*, (d) *Cunninghamella* sp., (e) *T. piluliferum* and (f) *P. chermesinum*



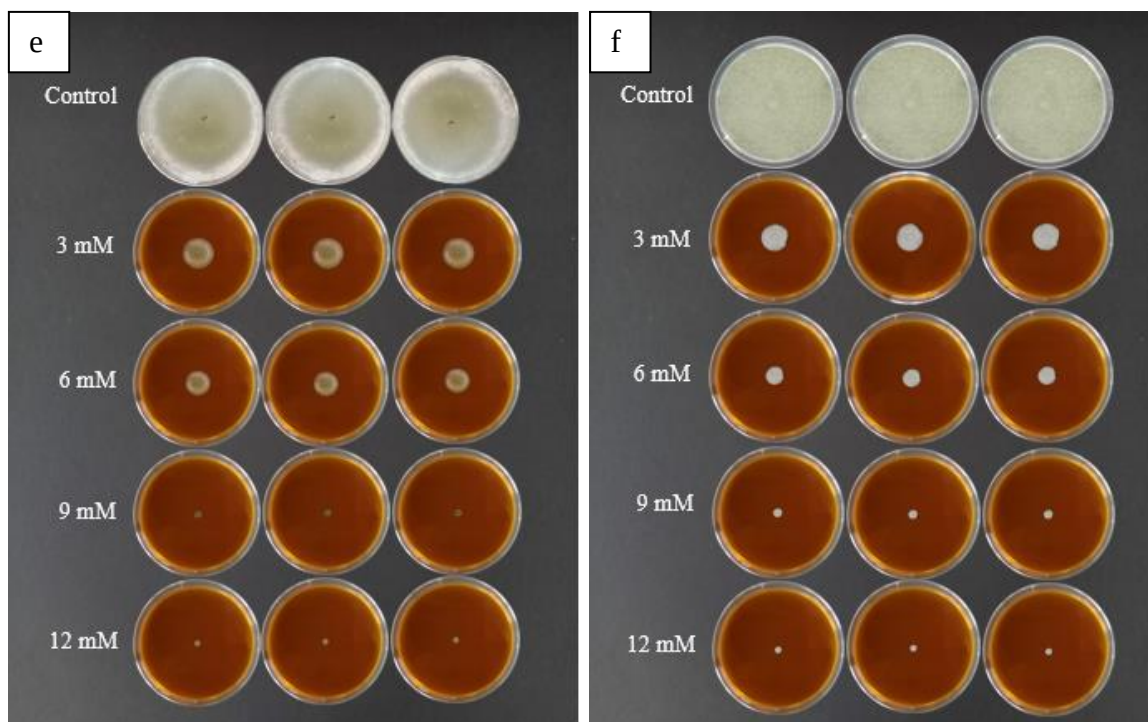


Figure 2: Macroscopic Image of Fungi Treated With I-LNPs at 40 nm of Various Concentrations Against (a) *F. equiseti* (b) *A. niger* (c) *A. flavus*, (d) *Cunninghamella* sp., (e) *T. piluliferum* and (f) *P. chermesinum*