INNOVATIVE STRATEGIES FOR RUBBERWOOD PRESERVATION: TEBUCONAZOLE LOADED FORMULATIONS VIA NANOPRECIPITATION AND SELF-EMULSIFYING DRUG DELIVERY SYSTEMS

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LIST OF SCHEMES

LIST OF SYMBOLS

%	Percentage
°	Degree angle
°C	Degree celsius
g	gram
h	hours
J	joule
kV	kilovolt
L	litre
mins	minutes
ppm	parts per million
PSI	pounds per square inch
V	volume
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight
W_{AD}	air-dried weight
W_{OD}	oven-dried weight
λ	wavelength
µm	micrometre

LIST OF ABBREVIATIONS

ACQ	Alkaline copper quaternary
ACZA	Ammoniacal copper zinc arsenate
CA	Copper azole
CAZymes	Carbohydrate-Active Enzymes
CCA	Chromated copper arsenate
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
FTIR	Fourier transform-infrared
HLB	Hydrophilic-lipophilic balance
PEG 400	Polyethylene glycol 400
PODs	Peroxidases
ROS	Reactive oxygen species
SEDDS	Self-emulsifying drug delivery system
SEM-EDX	Scanning electron microscopy-electron dispersive x-ray
TEB	Tebuconazole
UV-Vis	UV-Visible spectroscopy
WHO	World Health Organisation
$Z_{average}$	average hydrodynamic size

LIST OF APPENDICES

STRATEGI INOVATIF UNTUK PENGAWETAN KAYU GETAH: FORMULASI BERMUATAN TEBUCONAZOLE MELALUI PENDEKATAN PEMENDAKAN NANO DAN SISTEM PENGHANTARAN DRUG PENGEMULSIAN KENDIRI

ABSTRAK

Pengawetan kayu adalah penting dalam melindungi kayu daripada kemerosotan, dan teknologi baru telah dikaji untuk meningkatkan penghantaran dan kelarutan bahan pengawet melalui sistem tekanan vakum. Dalam kajian ini, nanopartikel zein yang dimuatkan dengan tebuconazole (TEB-nanopartikel zein) dan sistem penghantaran drug pengemulsian kendiri (SEDDS) yang dimuatkan dengan tebuconazole (TEB-SEDDS) telah disintesis. Zein, sebagai bahan pembawa polimer nano, mempunyai keserasian biologi yang baik, sifat biodegradasi, dan kestabilan drug untuk membawa TEB, dan TEB-nanopartikel zein boleh disediakan melalui pendekatan pemendakan nano pH, menggunakan Tween 80 sebagai surfaktan. TEB-nanopartikel zein yang dioptimumkan disintesis dengan saiz hidrodinamik pada 146.4 ± 10.66 nm dengan kecekapan pemuatan 42.97 %. Sebaliknya, SEDDS terdiri daripada minyak sawit sebagai minyak, Tween 80 sebagai surfaktan, dan PEG 400 sebagai ko-surfaktan. Komponen-komponen ini tidak menganggu TEB, dan boleh membentuk emulsi secara spontan yang boleh mengkapsulkan TEB apabila dalam air. Formulasi SEDDS yang dioptimumkan dan kestabilan secara kinetik terdiri daripada 0.02% minyak sawit, 0.03% S_{mix}, dan 99.95% air. Saiz titisan hidrodinamik adalah 1669.0 ± 334.1 nm, dan pemulihan purata TEB adalah 110.6 ± 4.7 % menunjukkan kecekapan pemuatan yang baik. Kedua-dua pendekatan digunakan untuk merawat kayu getah (*Hevea brasiliensis*) melalui rawatan secara tekanan vakum sel penuh yang diubah suai, dan diuji untuk ujian pendedahan anai-anai bawah tanah (*Coptotermes gestroi*), kulat reput perang (*Gloeophyllum trabeum*) dan kulat reput putih (*Trametes versicolor*). Kedua-dua kaedah rawatan menghasilkan kayu yang mudah dirawat dengan pengekalan bahan kimia yang baik, dengan nilaian "sangat tahan" terhadap anai-anai. Kedua-dua pendekatan rawatan juga berkesan terhadap kulat reput coklat dan putih, mengurangkan kehilangan berat dalam sampel kayu yang dirawat dan mencapai penilaian "sederhana tahan" dalam ujian antikulat. Kaedah pengawetan TEB-nanopartikel zein dan TEB-SEDDS berkesan untuk meningkatkan penghantaran TEB, mengurangkan dos bahan awet dan kos rawatan kayu, serta menunjukkan penemuan baharu untuk memudahkan penyerapan bahan awet kayu. Pendekatan SEDDS meningkatkan penghantaran TEB dalam kayu getah lebih berbanding dengan nanopartikel zein, ditunjukkan dari peningkatan rawayan, pengekalan kimia, dan kesanan terhadap anai-anai dan kulat lebih baik. Dengan dos TEB yang dikurangkan sebanyak 0.0025 %w/v, SEDDS menunjukkan kecekapan yang lebih tinggi dan menyediakan penyelesaian yang lebih berkesan serta inovatif untuk pengawetan kayu, menawarkan potensi untuk aplikasi yang lebih luas dalam industri ini.

INNOVATIVE STRATEGIES FOR RUBBERWOOD PRESERVATION: TEBUCONAZOLE LOADED FORMULATIONS VIA NANOPRECIPITATION AND SELF-EMULSIFYING DRUG DELIVERY SYSTEMS

ABSTRACT

Wood preservation is important in protecting wood from deterioration, and new technology has emerged to improve current preservative delivery and solubility under the vacuum pressure system. In this research, tebuconazole-loaded zein nanoparticles (TEB-zein nanoparticles) and tebuconazole-loaded self-emulsifying drug delivery system (TEB-loaded SEDDS) were synthesised. Zein, as a polymeric nanocarrier material, has good biocompatibility, biodegradability, and drug stability to incorporate TEB, and TEB-loaded zein nanoparticles can be prepared via the pH nanoprecipitation approach, using Tween 80 as a surfactant. Optimised TEB-loaded zein nanoparticles were synthesised with hydrodynamic particle sizes at 146.4 ± 10.66 nm with 42.97 % of loading efficiency. On the other hand, the SEDDS consists of palm oil as oil, Tween 80 as surfactant, and PEG 400 as co-surfactant. These components do not interfere with TEB and can form an emulsion spontaneously which can encapsulate TEB when in water. The kinetically stable optimised SEDDS formulation consisted of 0.02% palm oil, 0.03% S_{mix}, and 99.95% water. The hydrodynamic droplet size was 1669.0 ± 334.1 nm, and the average TEB recovery was 110.6 ± 4.7 %, indicating good loading efficiency. Both approaches were used to treat rubberwood (*Hevea brasiliensis*) *via* modified full cell vacuum pressure treatment and was tested for anti-termite and antifungal assays against subterranean termites (*Coptotermes gestroi*), brown rot (*Gloeophyllum trabeum*) and white rot fungi

(*Trametes versicolor*). Both approaches resulted in easily treatable wood with good chemical retention, and the treated samples were rated as "highly durable" against termites. Both approaches were effective against brown and white rot fungi, significantly reducing weight loss in treated wood samples and achieving a "moderately resistant" rating in the antifungal test. Comparing both approaches, the SEDDS approach significantly improves TEB delivery in rubberwood compared to zein nanoparticles, as shown by enhanced treatability, chemical retention, and superior anti-termite and antifungal activity. With a reduced TEB dosage of 0.0025 %w/v, SEDDS demonstrates higher efficiency and provides a more effective, innovative solution for wood preservation, offering potential for broader applications in the industry.

CHAPTER 1

INTRODUCTION

1.1 Overview

Wood is an organic, sustainable, and renewable resource used across diverse sectors such as construction and furnishing. Wood also plays an important role in carbon capturing to establish environmental equilibrium, and is capable of being reused after its life cycle to be converted into biomass (Kumar Sarangi et al., 2023). Wood has great constructional value as it has high strength-to-weight ratios, good mechanical strength and workability, and good insulation (R. Wang & Haller, 2022). Its versatile usability has allowed it to replace traditional construction materials and provide ecological benefits to construction. Malaysia being one of the largest timber-producing countries in the world, benefited from exporting RM21.14 billion worth of timber in 2015 (Ashaari, 2017). Recent reports of exported wood products amounted to USD 5.43 billion in 2020 (Latib et al., 2021). Rubberwood (*Hevea brasiliensis*) is a primary source of latex production, pulp, and paper manufacturing, contributing largely to wooden furniture as well. The revenue from rubberwood products in 2004 was reported to be as high as RM 6.5 billion (Teoh et al., 2011). Rubberwood's fibre dimensions, including optimal length and diameter, allow for efficient utilisation in these applications (Onakpoma et al., 2023).

Rubberwood and other wood species are mainly composed of cellulose, lignin, and hemicellulose. These components are hygroscopic and uptake water, which makes them vulnerable to deterioration and degradation caused by biotic and abiotic factors (Anagnost, 2007; Dimou et al., 2017; Marais et al., 2022). Biotic factors include damage caused by fungi, termites, or mould, whereas abiotic factors include environmental factors like temperature and humidity. These factors will affect wood's structural integrity, functionality, and overall service longevity (Woods & Watts, 2019). Hence, the wood industry has to preserve wood from degradation and decay from the impacts of biotic and abiotic damage to prolong the service life of wood-based products, where biotic damage impacting wood durability more (Marais et al., 2022; van Niekerk et al., 2021).

In the wood industry, wood preservatives are chemicals used to protect wood from decay and degradation due to biotic and abiotic factors, depending on its composition (Barbero-López et al., 2021; Khademibami & Bobadilha, 2022; Li et al., 2024). The application of wood preservatives has found widespread use as many types of wood preservatives have developed using different materials which can be categorised as oil-borne, water-borne, and others. Oil-borne preservatives are one of the oldest preservatives that contain tar and heavy solvents (Lebow, 2010). They were initially applied to wood as they showed protective properties but were soon discovered to harm the environment and health due to the inclusion of many toxic components and heavy metals (Kraševec et al., 2021; Tetenbaum-Novatt, 2024). Water-borne preservatives soon replaced oil-borne preservatives including copper which displayed good antimicrobial and antifungal effects (Lebow, 2010). However, some copper-based preservatives, often accompanied by chromium and arsenic, were detrimental to health and the environment (Mohajerani et al., 2018; Jones et al., 2019). One of the recently researched wood preservative types is organic biocides, which have a less harmful health and environmental impact while protecting against fungi and termites. Organic biocides include azole-based biocides, isothiazolinones and boron acid groups (S. R. Shukla et al., 2019; Silva et al., 2020; Zhang et al., 2023). The triazole group consists of organic biocides which are good fungicides that can disrupt fungi growth, which has found application as antifungal agents. Tebuconazole (TEB) is a triazole organic fungicide that has exceptional antifungal properties against white and brown rot fungi, and has been used for crop protection (de Albuquerque et al., 2018). However, its poor solubility in water disallows it to be directly applied to wood using industrial standards, where preservatives are applied through a water medium (Li et al., 2017). By using different methods using different solvents, chemical modification to the wood preservative, or nanotechnology, can improve the solubility and delivery of wood preservatives for treatment applications.

Nanotechnology has been studied extensively to be used for efficient carriers of active ingredients such as drugs and essential oils (Afzal et al., 2022; Nair et al., 2022), and it can be a potential approach to transport poorly soluble wood preservatives. One approach may be encapsulation in nanoparticles which improves drug delivery efficiency, as it protects the hydrophobic active ingredient from degradation or loss in the aqueous medium (Nair et al., 2022). In the wood preservation context, nanoparticles can penetrate the wood better due to their smaller and uniform sizes, enabling better delivery into the wood and providing sufficient protection (Paul et al., 2023; Bansal et al., 2024). Using the nanoprecipitation approach to prepare nanoparticles allows low-cost and simple design of the nanoparticles, as well as the upscale potential to produce the nanoparticles in large amounts using cheaper and less extreme solvents (Wu et al., 2022). The nanoprecipitation approach can be prepared by natural polymers like zein, chitosan and alginate (Łętocha et al., 2022; Guadarrama-Escobar et al., 2023) and synthetic polymers like methyl methacrylate and polyethylene glycol (Miller et al., 2023), which offers versatility in nanoparticle design as well (Iván Martínez-Muñoz & Elizabeth Mora-Huertas, 2022). Zein, a naturally sourced corn protein, is a biodegradable and amphiphilic natural polymer that has been chosen for the preparation of nanoparticles in many studies due to its excellent biocompatibility (Hu & McClements, 2014; Campos et al., 2015).

Another approach implements self-emulsifying drug delivery systems (SEDDS) for the encapsulation of the wood preservative. SEDDS is composed of oil, surfactants (and co-surfactants if necessary), and water to form an oil-in-water (o/w) emulsion with fine droplets (Mahmood & Bernkop-Schnürch, 2019). SEDDS can encapsulate the hydrophobic active ingredient into droplets, which can be transported in the water medium into the wood matrix, improving the delivery efficiency. Palm oil contains medium to long-chain triglycerides, which enable emulsification easily, as well as provide stability and physicochemical stability for the encapsulated drug (Goon et al., 2019). Tween 80, a non-ionic, high hydrophilic-lipophilic balance (HLB) valued surfactant, provides low toxicity and good emulsification properties for the formulation, which performs well in emulsifying palm oil (Gomes et al., 2018). To enhance the emulsion formation, a co-surfactant can be used to improve the emulsion stability as well as the active ingredient solubility. A good choice of co-surfactant is polyethylene glycol (PEG) 400, which synergises with Tween 80 to emulsify palm oil (Miksusanti et al., 2023).

To overcome the poor water solubility of TEB, we can improve its solubility by incorporating TEB into nanocarriers to be delivered into the wood matrix through the water medium. Using nanotechnology, we propose two approaches: incorporation into nanoparticles via the nanoprecipitation method, or encapsulation into a SEDDS formulation. The current study aims to develop two new approaches to delivering TEB into rubberwood, by incorporating TEB into zein nanoparticles via the nanoprecipitation approach, and the encapsulation of TEB into (palm oil/Tween

80/PEG 400)-SEDDS approach. Optimisation of the two approaches shall be investigated, followed by characterisation using microscopic and spectroscopic methods. By using the two approaches mentioned above, improvement in the delivery efficiency of TEB into rubberwood, reduced TEB concentration required for treatment, as well as better antifungal and anti-termite performance in the biological efficacy tests are expected.

1.2 Problem statement

Most current wood preservatives are water-borne preservatives, with copper as the main component, such as ammoniacal copper zinc arsenate, acid copper chromate, and chromated copper arsenate. The presence of other toxic heavy metals, such as chromium and arsenic in these biocides cause serious health and environmental implications. To mitigate this problem, organic biocides are used to protect wood against biotic damage to replace the use of copper-based biocides. Tebuconazole (TEB), an organic triazole fungicide, has been recommended as an alternative for wood protection. However, TEB has poor water solubility, which cannot be employed in the industry standard vacuum pressure treatment to be incorporated into the wood matrix, as the vacuum pressure method requires the wood preservative to be delivered into the wood by immersing the wood in water and force the wood preservative into the wood through pressure. To overcome these shortcomings, two approaches are proposed to improve the solubility of TEB in water and subsequently improve the delivery efficiency of TEB in water. The first approach is to prepare TEB-loaded zein nanoparticles via nanoprecipitation; as zein is an amphiphilic and biocompatible material, using the nanoprecipitation approach can easily synthesise nanoparticles to incorporate TEB and improve its solubility in water. The second approach is to formulate a self-emulsifying drug delivery system (SEDDS) to encapsulate hydrophobic TEB in the formulation. Using a medium and long chain-rich oil such as palm oil, a high hydrophilic-lipophilic balance (HLB) valued surfactant such as Tween 80, and a co-surfactant to improve the stability of the emulsion, such as polyethylene glycol 400, a stable emulsion can be prepared to encapsulate TEB in the emulsion droplets when dispersed in the water medium, improving the solubility and delivery efficiency of TEB. Using the two approaches mentioned above, TEB is expected to be delivered more effectively with lowered concentration into the rubberwood matrix and will perform better than conventional treatment of wood using TEB.

1.3 Objectives

The objectives of this study are:

i. To synthesise TEB-loaded zein nanoparticles and TEB self-emulsifying formulation via nanoprecipitation and self-emulsifying drug delivery system techniques and subsequently optimising the two approaches.
ii. To characterise the TEB loaded-zein nanoparticles and TEB-loaded SEDDS incorporating tebuconazole using microscopic and spectroscopic methods.
iii. To study the antifungal and anti-termite properties of TEB-loaded zein nanoparticles and TEB loaded-SEDDS on rubberwood (*Hevea brasiliensis*) as model wood.

1.4 Thesis outline

This thesis consists of five chapters:

CHAPTER 1 is the overview of the study. It focuses on the problem statements, research objectives, and outline of the contents.

CHAPTER 2 is a detailed literature review on wood structure, wood degradation, wood preservation, tebuconazole, nanotechnology and its applications, the nanoprecipitation approach with zein, and the self-emulsifying drug delivery system approach.

CHAPTER 3 elaborates on the general methodology of the study. It explains the methods for preparing zein nanoparticles via the nanoprecipitation approach and the formulation of SEDDS. The optimisation methods for both approaches were also discussed. The basic principles of the instrument characterization methods by Fourier transform-infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), dynamic light scattering (DLS), scanning electron microscopy and energy dispersive X-ray (SEM-EDX), were explained. The use of ultraviolet-visible (UV-Vis) spectroscopy, as well as the validation for determination of TEB concentration, were described. The vacuum pressure treatment for rubberwood samples, calculation of treatability, chemical retention and leaching studies, as well as the biological efficacy tests for resistance against fungi and termites were described.

CHAPTER 4 discusses the synthesis, optimisation and characterisation results obtained for the zein nanoparticles and the TEB-loaded zein nanoparticles via nanoprecipitation approach. Treatability, chemical retention and leaching studies of nanoprecipitation approach, followed by the biological efficacy performance of TEB loaded-zein nanoparticles treated rubberwood against fungi and termites were discussed.

CHAPTER 5 discusses the formulation, optimisation and characterisation results obtained for the (palm oil/Tween 80/PEG 400) SEDDS and the TEB-loaded (palm oil/Tween 80/PEG 400) SEDDS. Treatability, chemical retention and leaching studies of the SEDDS approach, followed by the biological efficacy performance of TEB loaded-SEDDS rubberwood against fungi and termites, were discussed.

CHAPTER 6 gives a conclusion to the study and valuable recommendations for future work.

CHAPTER 2

LITERATURE REVIEW

2.1 Wood

2.1.1 Wood anatomy

Wood can be categorised into two main types: hardwood and softwood. Hardwoods are derived from angiosperms (flowering plants) and contain vessel elements, fibres, and parenchyma cells. The presence of vessel elements, which form pores, often results in a more complex and variable structure (Wiedenhoft & Miller, 2005). Hardwoods are typically denser and more durable, making them suitable for applications like furniture, flooring, and high-load structures. Common examples include oak, maple, and rubberwood. Conversely, softwood is derived from gymnosperms (conifers); softwoods are predominantly composed of tracheids and resin canals with relatively uniform structures (Wiedenhoft & Miller, 2005). They lack vessel elements, which makes them less porous and more consistent in texture but include scaly cones that contain seeds. Softwoods like pine, spruce, and fir are often used in construction, paper production, and general-purpose applications.

Hardwoods and softwoods differ in their anatomy and primary cell types. Softwoods have a relatively simple structure composed of axial tracheids and parenchyma cells. Tracheids are long cells that occupy most of the softwood volume, giving mechanical support and transporting water in the wood. On the other hand, parenchyma cells include the axial and ray parenchyma cells. The axial parenchyma are vertically oriented cells that stack on each other to form a strand (Thomas, 1977).

In contrast, ray parenchyma cells appear brick-like and form in the radial direction intersecting with axial tracheids, which can act as food storage and provide lateral transportation of nutrients (Thomas, 1977). Another part of the softwood structure is pits that are spread out across the tracheid cell walls to allow water flow to move through cells, and also the conduction of wood in rough conditions (Esteban et al., 2023). Resin ducts also exist in longitudinal and radial configurations that secrete oleoresin to repel insects and fungi (Chauhan et al., 2022).

Hardwood structures also contain fibres and ray cells, but they can be differentiated from softwood as they feature vessels (Parham & Gray, 1984). Vessels are axial tubes that serve as the primary cellular channels that facilitate water and essential nutrients movement from the root system to the rest of the tree, similar to all axial cellular components present in the secondary xylem, enabling hardwoods to be more porous (Esteban et al., 2024). Vessels differ in segment ends depending on the different hardwood species and vary from 0.02 – 0.3 mm in diameter (Butterfield & Meylan, 1980; Parham & Gray, 1984). Hardwood fibres are elongated and aligned longitudinally with the tree trunk and play a significant role in enhancing the mechanical strength of the wood and its durability. Ray parenchyma cells are also present in hardwoods but in greater volume. They are wider than softwoods and serve identical functions given in softwoods. Figure 2.1 summarises the anatomical differences between softwood and hardwood. By understanding the differences in the structure of the wood, we can adjust our approach to preservative treatments of the wood.

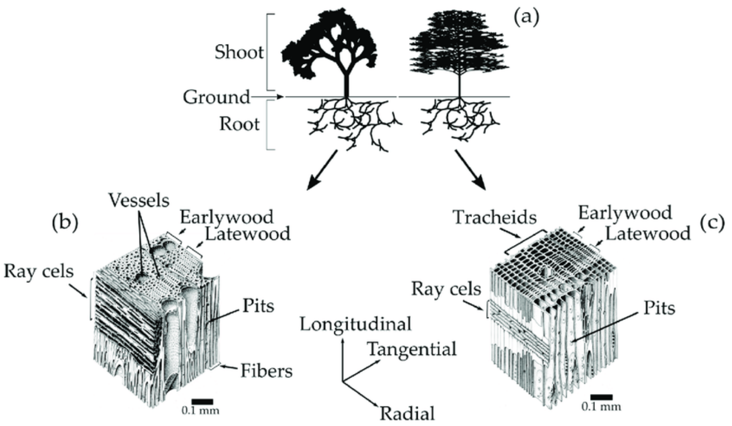

Figure 2.1 Illustration of a) general tree structure, and comparison of cellular structure between b) hardwood and c) softwood (Arzola, 2019)

2.1.2 Wood degradation

Wood degradation is a naturally occurring phenomenon that may be caused by biotic or abiotic pathways, as wood is inherently degradable. It contributes to the wood's structural integrity, resilience, and overall functionality. Biotic factors include attacks from fungi, termites, and other wood-damaging animals or insects. Aside from biotic damage, abiotic factors include environmental factors, temperature, and humidity, which can also cause degradation. Degraded wood will lose density, firmness, mechanical strength, and aesthetics, which will not be beneficial for commercial use.

Abiotic damage stems from non-biological factors and causes damage to wood. One of the most common sources of abiotic damage is moisture. Wood is a hygroscopic material, and the surrounding climate and humidity can influence its moisture content. Moisture content is responsible for dimension stability and stiffness, and in many cases, the high moisture content may lead to swelling, affect the radial and tangential dimensions of the wood and may ultimately cause cracks (Dietsch et al., 2015). Temperature is another abiotic factor which affects dimension stability.

Although thermal reactions below 110 °C do not affect the wood structure, heat energy can cause thermal expansion and contraction, which stresses the wood matrix and weakens the internal structure (Reinprecht, 2016a). Prolonged exposure to ultraviolet light causes photodegradation, resulting in surface discolouration and delignification (Srinivas & Pandey, 2012). Chemical exposure, particularly to acidic or alkaline substances from industrial pollutants or acidic fog, can cause structural breakdown and surface erosion (Williams, 1988). Weathering such as strong winds or rain contributes to surface degradation and erosion over time (Reinprecht, 2016b). These abiotic factors mentioned above affect the overall wood structure and quality which disallows it from further applications and processing.

Wood is decomposed mainly by two types of fungi: brown rot or white rot fungi and a minority of soft rot fungi. Both types of fungi damage the wood through different mechanisms: brown rot fungi attack cellulose in the wood while not engaging lignin, causing it to darken and become brittle (Sandberg et al., 2021). In contrast, white rot fungi decompose structural components like hemicellulose and lignin and pale the wood's colour (Sandberg et al., 2021). Brown rot fungi have a straightforward decay pattern, which utilises the Fenton reaction shown in Equation 2.1 to produce reduced metals such as Fe^{+} to form hydroxyl radicals ($HO^{\bullet}$) when reacted with hydrogen peroxide (H_2O_2), which depolymerizes and oxidises lignocellulose (Arantes & Goodell, 2014).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + HO^{\bullet} \qquad \text{Equation 2.1}$$

On the other hand, white rot fungi can produce simultaneous decay patterns. This is possible as white rot fungi utilise both enzymatic and oxidative pathways. As it can release a diverse array of ligninolytic enzymes like peroxidases (PODs) and utilize reactive oxygen species (ROS) to commence and perpetuate the degradation process (Mester et al., 2004). The interaction between these enzymes and ROS is essential for the efficient disintegrating of lignocellulosic constituents. *Tramates versicolor* is a common white rot fungus that can release PODs after penetrating through wood cell walls and pits, degrading lignin, cellulose and hemicellulose (Bari et al., 2019).

Insects, such as wood-boring beetles and termites, attack wood for food, shelter, and breeding purposes. Termites are well known to destroy wood, as they consume cellulose and lignocellulose and use it to create colonies and reproduce. Three main categories of termites are drywood termites, subterranean termites, and dampwood termites. In contrast to drywood and dampwood termites, which inhabit the timber they infest, subterranean termites establish colonies beneath the ground or within moist, sheltered habitats (Chouvenc et al., 2016). These termites construct intricate tunnel networks that extend from their nests to wood sources, enabling them to consume the wood while simultaneously remaining shielded from external threats (Noirot & Darlington, 2000). *Coptotermes gestroi*, a species of subterranean termites, produces a variety of carbohydrate-active enzymes (CAZymes) that facilitate the breakdown of cellulose and hemicellulose. These include endo-glucanases and beta-glucosidases, which are crucial for lignocellulose degradation (Franco Cairo et al., 2016).

The inherent lignocellulosic nature of wood makes it susceptible to biotic and abiotic damage. Given the presence of moisture, oxygen and suitable temperature, fungi and termite attacks are unavoidable and will result in wood degradation. Degraded wood is hard to process and has low economic and practical value, hence protection is needed to ensure wood can be processed without the influence of biotic and abiotic damage.

2.2 Wood preservation

2.2.1 Wood preservatives

To protect the wood from biotic damage, wood preservation is needed to provide protection and preserve wood for further use. Existing wood preservation approaches, including chemical treatment, thermal treatment, and biocontrol agents, have been employed to preserve and extend the longevity of wood (Bi et al., 2021; Alorbu & Cai, 2022; Goli et al., 2023). One current approach that is heavily researched is the application of wood preservatives. Wood preservatives are chemical compounds that are utilised in the treatment of wood to improve its longevity and safeguard it against biological as well as environmental deterioration (Vani et al., 2022). Wood preservatives are instrumental in prolonging the lifespan of wood by mitigating damage caused by fungi, insects, and various other organisms. The selection of an appropriate preservative frequently depends on the intended use, environmental conditions, and specific risks associated with the wood. Compared to other wood preservation methods, the use of wood preservatives has some advantages, as it does not involve modifying wood with harsh chemicals, solvents or conditions, and it has versatile applications methods including pressure and non-pressure treatments, allowing flexibility in application (Tarmian et al., 2020). Wood preservatives can be divided into three main categories: oil-borne, water-borne, and others.

Oil-borne preservatives have been one of the oldest types of preservatives, with many of the components dispersed in an oil-based carrier to be delivered into wood. The choice of oil carrier includes vegetable oils and biodiesels, which can also act as a barrier to remove moisture and hinder fungi or termite growth (Sandberg et al., 2021). Coal-tar creosote and pentachlorophenol are two major preservatives in this group, which have found widespread use in the early 20th century on railroad ties and telephone poles in the US (Lebow, 2010). Creosote is a tar oil distillate obtained from the high-temperature carbonization of coal. Six main creosote components include aromatic hydrocarbons, phenolics, tar bases, aromatic amines, sulphur-containing heterocycles, and oxygen-containing heterocycles, all of which are effective against pests (Cheremisinoff & Rosenfeld, 2010). Pentachlorophenol can function as a general biocide, containing a phenol group with five chlorine atoms, and its synthesis often produces byproducts and impurities, such as polychlorinated phenols and dibenzofurans (Cheremisinoff & Rosenfeld, 2010). Pentachlorophenol is usually dissolved in organic solvents, and it has been produced as a sodium salt that can be used in water mediums (Cheremisinoff & Rosenfeld, 2010).

The components of creosote and pentachlorophenol mentioned above contain many carcinogens, which quickly become a concern as their application in wood negatively impacts the environment and human health. In the application of creosote to wood and its field storage, organic compounds and derivatives from creosote are emitted, while further emission can occur in the heating process (Gallego et al., 2008). Volatile components in creosote, such as naphthalene, acenaphthene and acenaphthylene, may also leach into water and affect aquatic environments from treated products (Konkler et al., 2020). Many side effects, such as skin rash, convulsions, kidney or liver problems, and even unconsciousness from exposure to the emissions, have been reported (Cheremisinoff & Rosenfeld, 2010). Pentachlorophenol also interferes with cell respiration causes biochemical disruption in the body and has been classified to be carcinogenic by the US EPA (EPA, 2000). While oil-based wood preservatives provide wood protection, their possible health and environmental hazards are not ideal for modern-day use. Many countries have restricted the use of these preservatives due to environmental impact as well as disposal concerns, and many current studies focus on remedial and safe removal of the preservatives (Agbo et al., 2011; Hebisch et al., 2020; Omo-Okoro et al., 2023). Hence, safer and less harmful alternatives are needed.

Water-borne preservatives are the counterpart of oil-borne preservatives, and they use water to act as the carrier for the preservatives. They have the potential to be safer alternatives relative to oil-borne preservatives. Water-borne preservative formulations primarily incorporate copper as it has excellent fungicidal performance and includes chromium and arsenic, demonstrating commendable efficacy during utilisation (Lebow, 2010). Water-borne preservatives are suitable for wood that requires painting and for use in railroads, building foundations, and lumber (Smith, 2020). Standard formulations include acid copper chromate, ammoniacal copper zinc arsenate (ACZA), and chromated copper arsenate (CCA) (Lebow, 2010; Khademibami & Bobadilha, 2022). These preservatives provided dimensional stability and resistance against fungi and termite attacks, which were more effective than oil-borne preservatives. Still, similarly, they contained heavy metals that were prone to leaching and posed environmental risks. Chromium and arsenate in CCA have adverse effects on the environment and human health, such as leaching and bioaccumulation, harming body parts like the brain, kidneys, and reproductive organs if exposed (Morais et al., 2021). Incineration of CCA or ACZA-treated wood would be possibly detrimental as chromium and arsenic can be diffused and inhaled by humans, leading to an increased risk of lung cancer (Ohgami et al., 2015). Therefore, CCA and ACZA have been used with restrictions for non-residential lumber applications to reduce the possibility of exposure to humans. Alternatives like copper azoles (CAs) and alkaline copper quaternary (ACQ) were developed, and the main biocide of both formulations is the copper salts in aqueous monoethanolamine. The difference in the formulations is the co-biocides: CA contains amine copper and tebuconazole (TEB) with some variations that also contain propiconazole, while ACQ includes quaternary ammonium compounds (Shukla et al., 2019).

As research progresses, more preservatives are developed with improved functions and decreased health and environmental risks. Recent preservatives such as triazoles, borates, pyrethroids, and essential oils have been reported and researched on their application against fungi and termite attacks (Barbero-López et al., 2021; Khademibami & Bobadilha, 2022). Specifically, triazoles are organic wood preservatives as they have excellent antifungal characteristics, which have found use in pharmaceutical fields (Peyton et al., 2015) and used to control fungal diseases worldwide (Cui et al., 2021). Triazoles generally have a five-membered ring with two carbon and three nitrogen atoms, one nitrogen atom more than imidazoles. They obstruct the biosynthesis of ergosterol, thereby interfering with the formation and growth of fungal cell membranes (Houšť et al., 2020; Shafiei et al., 2020). Triazoles can hinder the synthesis of ergosterol, resulting in membrane destabilization of the fungal cells, which deters their growth on wood (Marzi et al., 2022). Organic wood preservatives like triazoles are biodegradable and can be deactivated through incineration, which presents an environmentally friendly alternative to previous preservatives (Palanti & Susco, 2004).

2.2.2 Tebuconazole (TEB)

TEB is a triazole fungicide with a chemical name 1-(4-chlorophenyl)-3-(1,2,4-triazol-1-yl)-2-(1,1-dimethylethyl)-2-propanol, is highly effective against fungi used for cereals, vegetables, and fruits (Želonková et al., 2019; Dong, 2024), as the structure is shown in Figure 2.2. TEB has a triazole functional group, which is responsible for its fungicidal activity. Compared to other preservative predecessors, TEB is entirely composed of organic elements without heavy metals, which provides a safer alternative. The mechanism of TEB involves the inhibition of ergosterol synthesis, which subsequently influences the integrity of fungal cell walls and leads to the suppression of fungal growth (Dong, 2024). This allows TEB to show antifungal activity against brown rot, white rot, and soft rot fungi. As mentioned, TEB also acts as a co-biocide in copper azoles and their derivatives, which are used as antifungal preservatives.

TEB can be less utilised in aqueous preservative formulations designed for pressure treatments, and it constitutes one of the active constituents within light organic solvent preservatives, as TEB is soluble in some organic solvents but has poor solubility in water (Li et al., 2017). The World Health Organization (WHO) labelled TEB with the second category of toxicity, indicating a moderate degree of hazard, with a lethal dose (LD_{50}) quantified at 1700 mg/kg (WHO, 2019). Even so, TEB was studied for its degradation and showed fair results with the potential to lower environmental impact (Cabras et al., 1997; Obanda et al., 2008).

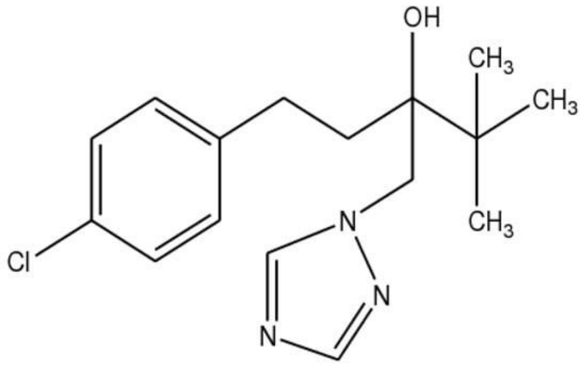

Figure 2.2 Structure of tebuconazole (TEB) (Želonková et al., 2019)

TEB application on wood in the past has shown promising results against fungi. The earliest studies show the application of TEB with heated oil to have a one-step treatment of the preservative and the oil carrier into Scots pine wood blocks (Palanti & Susco, 2004). Later, newer approaches to delivering TEB have been researched, such as incorporation into the supercritical fluid (Acda et al., 2001), naphtha-based solvent (Kukowski et al., 2016) and forming metal complexes with zinc and copper (Evans et al., 2007). More recent research highlights nanoparticles (Campos et al., 2015; Xu et al., 2020; Shi et al., 2022) and emulsion delivery approaches (Díaz-Blancas et al., 2016; Lucia et al., 2021), which show promising results as they are more efficient, indicating better penetration and retention in these studies. However, it is essential to be aware that treatment approaches depend heavily on the wood species, porosity, homogeneity, and surface roughness (Shupe et al., 1998). Hence, careful selection of the wood preservative application method is crucial to allow the treatment approach to be effective on wood.

2.2.3 Wood preservative application methods

Wood preservatives are applied to wood mainly through pressure and non-pressure methods, allowing the preservatives to fixate on the wood and provide its protection. Pressure methods utilise high pressure to force the preservatives into the wood matrix. Wood is generally placed into a cylindrical container and filled with preservatives, which can be oil-borne or water-borne. Pressure is then applied to the wood to force the preservatives to be absorbed into it until the desired level of treatment is achieved. One more vacuum step is applied to remove excess preservatives on the wood surface, and the wood is retrieved to dry and taken for post-treatment or conditioning, depending on its end use (Lebow, 2010). Pressure methods can achieve deeper penetration into the wood as an external force is applied to the preservative to penetrate deep into the sapwood, and this can be applied for large-scale treatments. However, pressure treatment processes have high operating costs and require high energy, which may not be suitable for small-scale or batches of wood treatments.

Three pressure processes that are commonly found are the full cell, modified full cell, and empty cell methods (Teng et al., 2018). An example of the overall process is illustrated in Figure 2.3. The differences between the methods are based on the preservation concentration and the vacuum application. In the full-cell method, there is an initial vacuum step to remove air from the wood and a final vacuum step to dry the wood from excess preservatives. The modified full-cell method uses lower levels of initial vacuum, which depends on the treated wood species and the preservative level required (EPA, 1999). The empty cell method is commonly applied for oil-borne preservatives and consists of the Rueping and Lowry processes. The Rueping process starts with an initial pressurisation period before preservative insertion in the cylinder (EPA, 1999). The Lowry process is like the Rueping process but applies preservatives to the wood without initial pressurisation (EPA, 1999).

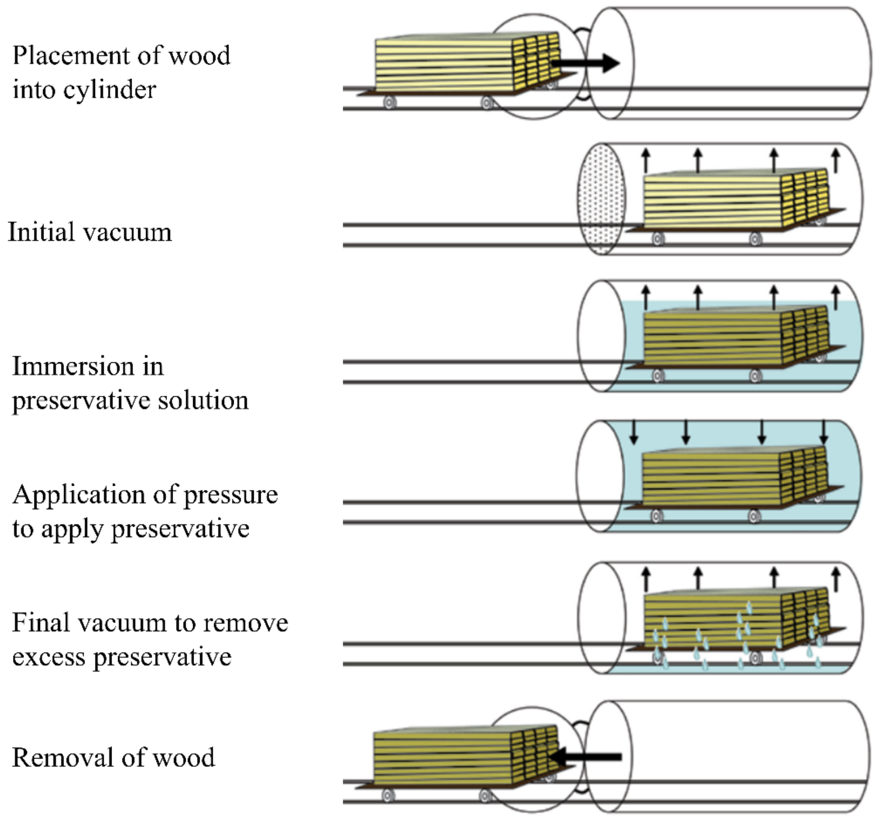

Figure 2.3 General steps of the pressure treatment process (Lebow, 2010)

Other than pressure methods, non-pressure methods can be used to protect wood superficially to give temporary protection when the wood species is not suitable for pressure treatments. Non-pressure methods include spraying, brushing, dipping, cold soaking, and hot-and-cold baths, which allow surface protection of the timber (Tarmian et al., 2020), which are illustrated in Figure 2.4. It is important to ensure preservative treatment in wood by choosing appropriate treatment methods and ensuring the wood preservative is retained in the wood after treatment. As the standard treatment medium is water, hydrophobic preservatives can hardly be treated in wood, while hydrophilic preservatives may pose leaching problems (Teng et al., 2018). Hence, optimising treatment methods and delivering preservatives are crucial to improving overall wood preservation. In contrast to the pressure treatment methods, non-pressure methods can be easily prepared and require lower energy and cost to operate. Still, the penetration of the wood preservatives may only be superficial and inconsistent in its application. This may further result in preservative leaching. Hence, non-pressure treated wood may not be suitable for heavy-duty applications such as utility poles, as they are more likely to be exposed to weathering and decay.

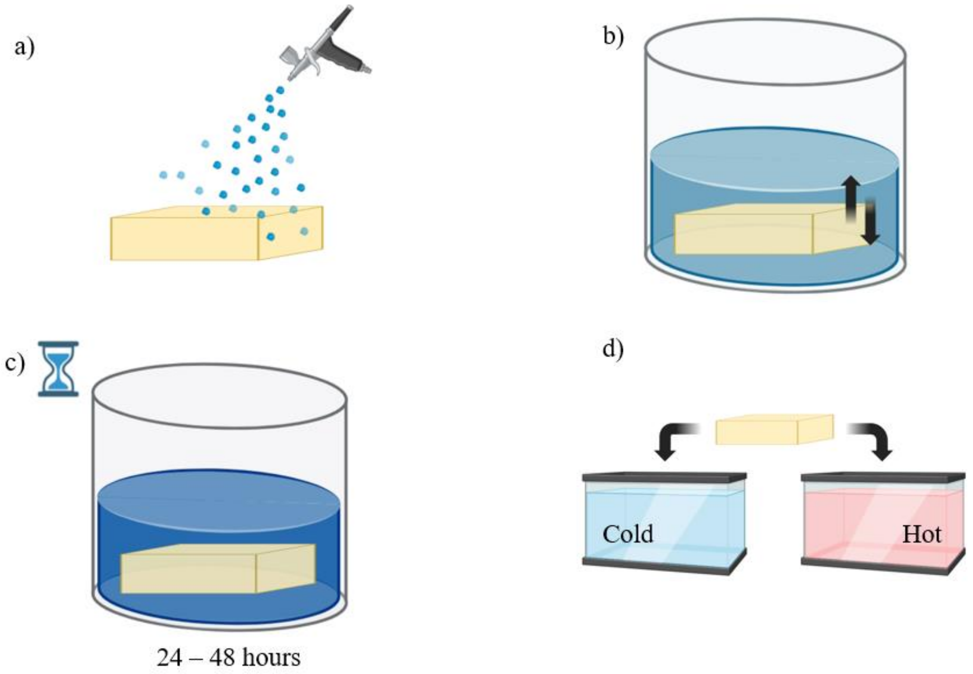

Figure 2.4 Illustration of examples of non-pressure methods: a) spraying, b) dipping, c) cold soaking, and d) hot-and-cold bath

2.3 Nanotechnology and application in wood preservation

Nanotechnology is crucial across numerous scientific disciplines at the forefront of generating novel opportunities within contemporary research, particularly in wood utilisation and preservation. This field involves the study of matter at atomic and molecular dimensions with structures smaller than 100 nm (Yusuf et al., 2023). Innovation in nanotechnology has found applications across different fields, such as medicine, electronics, and environmental science (Saravanan et al., 2021; Afzal et al., 2022; Samuel et al., 2022).

Nanotechnology introduces innovative solutions to protecting wood from biological degradation and environmental damage through nanomaterials. Nanomaterials, like nanoparticles, can allow for deeper penetration of preservatives, have a high surface area-to-volume ratio, and improve these treatments' durability, efficacy, and environmental friendliness, which improves wood protection (Papadopoulos & Taghiyari, 2019; Rahayu et al., 2023). Furthermore, studies have shown that nanomaterials have lower ecological effects while still providing comparable results with traditional preservative treatments. Still, the use of nanotechnology in wood science has been rising in the past decade, which has advanced the understanding of its potential uses.

2.3.1 Polymeric nanoparticles

Nanocarriers are nanomaterials that can act as carriers to transport a target substance to a certain location, and they have been found to have practical use in the pharmaceutical and electronic fields (Thakuria et al., 2021). Polymeric nanoparticles are one type of nanocarrier composed of monomer units that can transport drugs as well as wood preservatives. Polymeric nanoparticles have many characteristics of a good carrier: simple design, versatile base polymer materials, and smaller and more uniform sizes, thereby improving encapsulation and delivery of wood preservatives (Crucho & Barros, 2017; Borges et al., 2018). Furthermore, polymeric nanoparticles can be prepared from natural or synthetic polymers, to enhance the compatibility of the target preservative to the nanoparticle and improve delivery.

Polymeric nanoparticles can come in nanocapsules, nanospheres, nanomicelles, dendrimers and polymersomes (Teng et al., 2018). Wood preservatives that have low solubility can be disseminated in aqueous environments by the solid polymeric nanoparticles before the pressure water-borne treatment process. This allows wood preservatives to be delivered into the wood through the water medium. The polymeric nanocarrier enhances the infiltration of the wood preservative as it encapsulates or incorporates the hydrophobic active biocide from excessive leaching of the organic biocide (Bi et al., 2021). This allows hydrophobic wood preservatives to be employed in the industry standard vacuum pressure treatments, where water mediums are the norm for delivering the wood preservatives.

Polymeric nanoparticles can be prepared through two main strategies: bottom-up or top-down. Bottom-up approaches involve building nanoparticles from smaller components, such as monomers, through self-assembly. These include solvent evaporation, emulsion polymerisation, and nanoprecipitation (Bilati et al., 2005; Rao & Geckeler, 2011; Sahoo et al., 2021). For bottom-up approaches, solvent and non-solvent systems are commonly used to dissolve the polymer and induce nanoparticle precipitation in the non-solvent system (Chan & Kwok, 2011). Bottom-up approaches have the benefit of requiring less energy input and having better control over polydispersity, but the multistep process and limited scalability (Chan & Kwok, 2011). Top-down approaches operate the opposite principle by breaking down macro or bulk polymers into nanoparticles. These include grinding/milling, physical vapour deposition, and laser ablation. Top-down approaches do not rely on solvents, and material monitoring is much easier (Abid et al., 2022). Top-down approaches are much more direct and rapid. However, it is energy-intensive and destructive, resulting in wastage and limited control over morphology (Abid et al., 2022). Therefore, the approach should be chosen carefully to obtain the desired nanoparticle product.

Polymeric nanoparticles can be synthesised from natural or synthetic polymers, each offering specific benefits for various applications. The choice of material depends