forests

MDPI

Article

Oil in Water Nanoemulsions Loaded with Tebuconazole for Populus Wood Protection against White- and Brown-Rot Fungi

Alejandro Lucia [1,2,3,*], Mónica Murace [3,4], Gastón Sartor [3], Gabriel Keil [4], Ricardo Cámera [5], Ramón G. Rubio [6,7] and Eduardo Guzmán [6,7,*]

1 Instituto de Ecología y Desarrollo Sustentable (INEDES, CONICET-UNLu), Ruta 5 y Avenida Constitución, Luján, Buenos Aires 6700, Argentina
2 Centro de Investigación en Sanidad Vegetal (CISaV), Facultad de Ciencias Agrarias y Forestales, UNLP, Calles 60 y 119, La Plata 1900, Argentina
3 Facultad de Ciencias Agrarias y Forestales, UNLP, Calles 60 y 119, La Plata 1900, Argentina; mmurace@gmail.com (M.M.); gnsartor@gmail.com (G.S.)
4 Laboratorio de Investigaciones en Maderas (LIMAD), Facultad de Ciencias Agrarias y Forestales, UNLP, Calles 60 y 119, La Plata 1900, Argentina; gabrieldkeil@yahoo.com.ar
5 Química Bosques S.A, Florencio Varela, Buenos Aires B1888, Argentina; ricardocamera@direth.com.ar
6 Departamento de Química Física, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain; rgrubio@quim.ucm.es
7 Instituto Pluridisciplinar, Universidad Complutense de Madrid, Paseo Juan XXIII 1, 28040 Madrid, Spain
* Correspondence: luciaalejandro@yahoo.com.ar (A.L.); eduardogs@quim.ucm.es (E.G.)

Abstract: Eugenol in water nanoemulsions loaded with tebuconazole appear as a very promising alternative formulations for wood protection against xylophagous fungi that are the main species responsible for different rots in wood structures. The dispersions as prepared and upon dilution (impregnation mixtures) were characterized by the apparent hydrodynamic diameter distribution of the oil droplets loaded with tebuconazole and their long-term stability. The impregnation mixtures were applied on wood of *Populus canadensis* I-214 clone by using a pressure-vacuum system, and the effectiveness against fungal degradation by *Gloeophyllum sepiarium* and *Pycnoporus sanguineus* fungi was determined. The retention of tebuconazole in wood was about 40% of the amount contained in the impregnation mixtures. The results showed that the impregnation process leads to a long-term antifungal protection to the wood, with the mass loss after 16 weeks being reduced more than 10 times in relation to the control (untreated poplar wood) and the reference wood (untreated beech wood).

Keywords: emulsions; eugenol; eco-sustainable; xylophagous fungi; nanocarriers; tebuconazole; encapsulation

Citation: Lucia, A.; Murace, M.; Sartor, G.; Keil, G.; Cámera, R.; Rubio, R.G.; Guzmán, E. Oil in Water Nanoemulsions Loaded with Tebuconazole for Populus Wood Protection against White- and Brown-Rot Fungi. *Forests* **2021**, *12*, 1234. https://doi.org/10.3390/f12091234

Academic Editors: Lina Nunes, Dennis Jones and Bruno Esteves

Received: 12 August 2021
Accepted: 9 September 2021
Published: 10 September 2021

1. Introduction

Timber, together with steel and reinforced concrete, are currently among the most commonly used structural materials for building large structures [1]. However, wood undergoes changes as result of seasonal variations due to temperature and relative humidity changes and by the action of biotic and abiotic agents, which lead to slow physical and chemical deterioration processes of the external surface of wood structures [2], with the deterioration associated with xylophagous fungi being accounted among the most common and aggressive [3].

Wood, a lignocellulosic compound mainly formed by cellulose, hemicelluloses and lignin (main components of the cell wall), is susceptible to bio-deterioration by fungi and bacteria, especially under high environmental humidity conditions. The destruction of the polymers forming the wood results in a fast worsening of their mechanical properties, which reduces its shelf life [4,5]. The durability of wood products can be improved by physical or chemical treatments. This makes of the seeking of effective strategies to enhance their resistance and durability against fungal activity with the minimal environmental a

very important challenge [6]. There are currently available a broad range of chemical treatments for enhancing the durability and, in turn, the shelf life of timber. These treatments mainly rely on the impregnation of the wood cells, which make them more resistant to decay, insects, weather or fire. The degree of protection obtained upon such treatments depends, among other parameters, on the nature of the preservative molecules, and their penetration and retention within the wood [6].

In recent years, significant efforts have been paid to the development and evaluation of new products for wood preservation, e.g., renewable polymers such as chitosan [7–9] or propolis [8,10–12]; metal and metal oxide nanoparticles [8,13–20] or essential oils [21–23]. In particular, the use of natural compounds (natural extractives, vegetable and essential oils, natural resins and waxes, or various biopolymers) as biocides open new avenues for the development of eco-sustainable wood protection technologies [24]. Seeking new botanical fungicides to develop formulations of low environmental impact, the activity of essential oils against different species of fungi has been being extensively studied [25]. Neverthless, only a few essential oil compounds have evidence their effectiveness wood decaying fungi [17], among them the phenolic compounds (eugenol, carvacrol or thymol) or the oxygenated compounds such as elemol and cinnamaldehyde [21,26]. The results reported by Pánek et al. [21] showed that the minimum effective concentration of essential oils evaluated for protection of filter papers against brown-rot fungus *Coniophora puteana* was 3.5% in weight, whereas the white-rot fungus *Trametes versicolor* are less affected for the active compounds contained in the essential oils. On the other hand, Chittenden and Singh [26] studied the antifungal activity of eugenol and cinnamaldehyde as a preservative for radiata pine wood against common wood inhabiting fungi, and found that blocks treated with 3% *w*/*v* eugenol without exposure to wet conditions had <1% weight loss upon exposure to three types fungi (*Oligoporus placenta*, *Coniophora puteana* and *Antrodia Xantha*). However, blocks exposed to water showed weight losses in the range of 13–23%. It should be stressed that several studies have pointed that the inclusion of natural products in aqueous formulation may reduce the harmful environmental impact of conventional formulations [27,28].

On the other hand, tebuconazole and propiconazole are widely used fungicides in agriculture [29,30], which are commonly incorporated into commercial formulations for the treatment of lumber used on the building of residential structures [6]. The use of tebuconazole, alone or in combination with other active compounds, has been proved to be very effective for wood protection against fungal deterioration [31,32]. This may be understood considering that tebuconazole interferes with the biosynthesis of the fungal cell membrane, inhibiting the formation of ergosterol [33], and hence limiting the fungal growth and sporulation [34], which in turn results on the death of this type of organisms [35]. On the other side, tebuconazole presents a moderate toxicity for mammals (LD_{50} of 1700 mg/kg in rats) [36], with their use being approved in combination with other active ingredients for wood preservation in different countries (USA, Chile, United Kingdom and New Zealand). These good properties have allowed the tebuconazole inclusion in different commercial products for wood preservation [37,38]. However, the tebuconazole presents a low-moderate solubility in water (36 mg/L at 20 °C), which makes it susceptible to be washed (leaching). This together with their moderate toxicity to soil organisms has led to its classification by European Union Directive 98/8/EC, concerning on the placing of biocidal products on the market, as a chemical product for wood preservation under periodic revisions of its application conditions [39]. This can be understood, in part, considering the possible environmental risks, especially for aquatic organism, when wood treated with tebuconazole (by pressure wood treatment) is used for building of docks. This makes necessary seeking new eco-sustainable formulations for the preparation of systems containing tebuconazole for wood protection.

The applicability of conventional pesticide for wood protection has been increased during the last twenty years, which has allows the preparation of new pesticide formulations with an optimized effectiveness, improved safety for mammals and other non-target organ-

isms, handling, and storage [40,41]. This has been possible as result of the current advances of the colloidal physico-chemistry, which offer many tools that can be exploited for enhancing the availability of active molecules with technological or biological interest [42–44]. Emulsions are probably accounted as one of the most widespread colloidal systems for the solubilization and encapsulation of active molecules, allowing the distribution of such molecules within surfactant stabilized liquid droplets dispersed in a second immiscible liquid [45–49]. In particular, the use oil-in-water nanoemulsions (o/w nanoemulsions), with the oil phase being an essential oil compound, stabilized by amphiphilic copolymers have been demonstrated as a promising strategy for preparing formulations for control different insect pests [44,47,48,50–52], allowing the incorporation of poorly water-soluble molecules within their oil core [47]. This work tries to exploit eugenol in water nanoemulsions stabilized by the amphiphilic copolymer poloxamer 407 (a triblock copolymer with a central lipophilic block of poly(propylene oxide) and two peripheral hydrophilic blocks of poly(ethylene oxide)) for the solubilization and dispersion of tebuconazole for preparing formulations to be used against the wood bio-deterioration induced by xylophagous fungi. It is expected that this type of formulations can reduce the environmental impact associated with the use of tebuconazole [24,53–55]. Furthermore, this type of formulations can help on the reduction of the toxicological risks and hazards associated with the handling and application of products containing tebuconazole, as well as on the minimization of the combustion risks during the storage process of the formulations due to the removal of the flammable organic solvents used in the preparation of commercial emulsifiable concentrates [56–58]. On the other side, the combination of natural based compounds, such as essential oils compounds, and conventional pesticides can result in favorable synergistic effects in relation to the ability of the single components for wood protection [27].

2. Experimental Section

2.1. Chemicals

Eugenol (4-allyl-2-methoxyphenol) with a purity $\geq$ 99% and poloxamer 407 were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Poloxamer 407 is a triblock copolymer which presents an average molecular weight of 12.600 kDa (4.4 kDa each polyethylene oxide block and 3.8 kDa the central polypropylene oxide one). The fungicide tebuconazole (TEB); α-(2-(4-chlorophenyl)ethyl)-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol (technical grade, 98% of purity) was supplied by Química Bosques S.A. (Buenos Aires, Argentina). All the chemicals were used as received without further purification. Chemical structures of eugenol, tebuconazole and poloxamer 407 are shown in Figure 1.

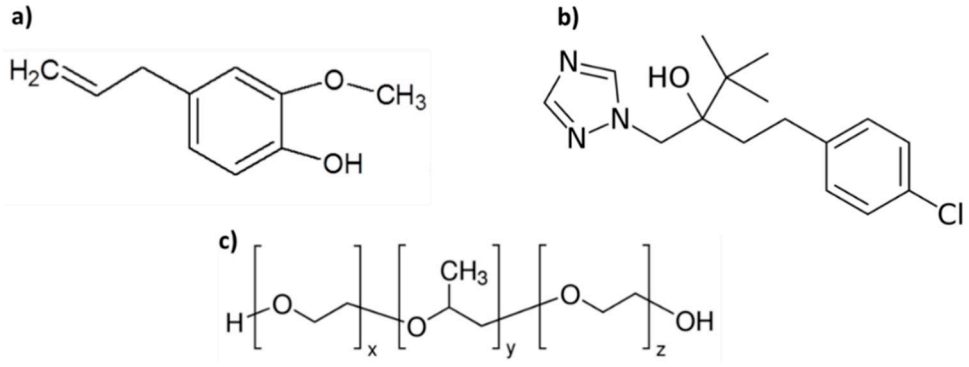

Figure 1. Chemical structures for eugenol (**a**), Tebuconazole (**b**) and poloxamer 407 with x = z = 101 and y = 56 (**c**).

The water used in this study was ultrapure deionized water (Milli-Q water) obtained from an AquaMAX^{TM}-370 Series multicartridge purification system (Young-Li Instrument, Co., Ltd., Gyeonggi-do, Korea), presenting a resistivity higher than 18 MΩ·cm and a total organic content lower than 6 ppm.

2.2. Preparation of Tebuconazole Carriers

The aqueous dispersion of tebuconazole solubilized in eugenol droplets stabilized by poloxamer 407 (eugenol in water nanoemulsions loaded with tebuconazole) were prepared in beakers of 100 mL following a procedure adapted from our previous publication [47], which can be summarized in the following steps: (i) the required amount of a stock solution of poloxamer 407 with concentration 10 wt% was weighted and poured into the beaker; (ii) the required amount of tebuconazole dissolved in eugenol is added to the beaker containing the copolymer solution, and (iii) the mixture is homogenized during 2 h under mild shaking using a magnetic stirrer (1000 rpm). It is worth mentioning that the inclusion of tebuconazole makes the homogenization difficult at 25 °C, thus it was needed to carry out the homogenization step at 60 °C to favor the tebuconazole solubilization.

The formulations used for solubilization and encapsulation of tebuconazole (1.5 wt%) contain poloxamer 407 and eugenol in concentrations of 7.5 wt% and 4 %wt, respectively. This results in a ratio between the concentrations of eugenol and poloxamer 407 < 0.8, which corresponds to the one-phase region of the pseudo-ternary water-eugenol-pluronic 407 system [47]. However, the inclusion of tebuconazole may push out the mixture from the one-phase region to the dispersions.

2.3. Determination of the Density of the Dispersions

The density of the dispersions was determined by weighing 0.5 and 1 mL aliquots of the dispersions containing tebuconazole at 25 °C using an analytical balance (AUY220, Shimadzu Corporation, Kyoto, Japan). The aliquots of the dispersions were taken using a Gas tight™ syringe (Hamilton Company, Reno, NV, USA). This procedure was repeated 5 times for each volume measured, and the density was determined as the average value. Measurements of the densities of the water, poloxamer 407 solution (7.5 wt%), and eugenol in water emulsion without tebuconazole were used for the sake of comparison.

2.4. Dynamic Light Scattering

Dynamic light scattering (DLS) experiments were carried out using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). The experiments were performed at 25 °C in quasi-backscattering configuration (scattering angle, θ = 173°) using the radiation from the red line of a He-Ne laser (wavelength, λ = 632 nm). The samples were transferred for the measurement to a quartz cell (model 6030-OG l, Hellma®, Müllheim, Germany). In DLS experiments, the normalized intensity autocorrelation function for the diffusion of Brownian scatters, $g^{(2)}(q,t)$ is obtained:

$$g^{(2)}(q,t) - 1 = \beta \exp(-t/\tau) = \beta \exp(D_{app} q^2 t), \quad (1)$$

where t and τ are the time and the characteristic diffusion time, respectively; $q = (4\pi n/\lambda) \sin(\theta/2)$ is the wavevector, with n being the refractive index of the continuous phase of the dispersion and D_{app} is the apparent diffusion coefficient. In Equation (1), β is an optical coherence factor which, in most of the cases, assumes a value close to 1. From the values of D_{app} it is possible to estimate the apparent hydrodynamic diameter of the scatters, d_h^{app}, through the Stokes-Einstein equation:

$$d_h^{app} = \frac{k_B T}{3\pi\eta D_{app}} \quad (2)$$

where k_B and T refer to the Boltzmann constant and the absolute temperature, respectively, and η is the viscosity of the solvent. It is worth mentioning that DLS can be only used with transparent one-phase dispersions.

DLS was used for the characterization of the dispersions of tebuconazole carriers (stock dispersions) as were prepared, and after 3 and 6 months of storage at 5 and 25 °C. Furthermore, DLS was also use for the characterization of the impregnation mixtures obtained upon dilution of the stock dispersions after being stored at two different temperatures

during different times. Impregnation with two different final tebuconazole concentrations (0.375 mg/mL and 0.750 mg/mL) were studied.

2.5. Impregnation of Wood with the Antifungal Compound by Vacuum-Pressure Treatment

Wood boards cut from the trunks of ten 10-year-old poplar trees (*Populus canadensis* Moench or *Populus euroamericana* (Dode) Guinier I-214 clone) located in the town of Pomona (Rio Negro, Argentina) were received as beams (50 × 150 × 2400 mm^3; density 400 kg/m^3) and conditioned until the hygroscopic equilibrium was reached, which was verified using a digital hygrometer (Gann HT 85, Gann Mess- u. Regeltechnik GmbH. Gerlingen, Germany). This step involved placing the block in a drying oven with a relative humidity of 60 ± 2% at 27 ± 2 °C during at least 10 days to reach an equilibrium moisture content corresponding to the 12% of the weight of the sample.

Once the hygroscopic equilibrium was achieved, samples with dimensions 20 × 20 × 160 mm^3 were cut from the original blocks. A sample of this wood was impregnated by vacuum-pressure treatment using autoclave and the rest was used as control material. The impregnation process was performed with mixtures containing two different concentrations of tebuconazole (0.375 and 0.750 mg/mL, identified as impregnation mixtures 1, IM1, and 2, IM2, respectively). These impregnation mixtures were obtained upon dilution with water of the stock dispersions.

The vacuum-pressure treatment was performed based on the modified Full-Cell process [59], which relies in the following steps: (i) wood blocks are placed in a tray and introduced into a stainless-steel autoclave; (ii) the impregnation mixture (at room temperature) was placed in the tray to cover all the wood; (iii) wood blocks are sealed in the autoclave and a preliminary vacuum (650 mmHg) is applied for 10 min to remove the air from the cylinder and as much air as possible from the wood; (iv) pressure is increased to 3 kg/cm^2 for 15 min; (v) pressure is finally removed; (vi) a short final vacuum (650 mmHg during 15 min) was used to remove dripping preservative from the wood, and (vii) the wood blocks are extracted from the remaining liquid. After impregnation, wood blocks were left during 24 days at 27 ± 2 °C and 60% relative humidity (RH) until constant weight in a conditioning chamber.

2.6. Efficacy against White- and Brown-Rot Fungi

The efficacy of the formulation was determined according to EN 113-1:2020 standard, which provides technical specifications about methodologies that can be used for evaluating the durability of wood and wood products against wood-destroying fungi, and for assessing the biocidal efficacy of wood preservatives. [60]. For the aim of this work, the specifications of the standard were modified in terms of the size of wood samples and xylophagous strains as was proposed by several authors [61–64]. Treated and untreated *Populus canadensis* I-214 clone wood samples, referred to as test material (TM), with dimensions 20 × 20 × 20 mm^3 and free of defects were used for biodegradation assays. *Fagus sylvatica* L. (beech) specimens, with similar characteristics to the TM, were used as reference material (RM) for the validation of the assays. 240 samples, which were previously oven-dried at 30 ± 2 °C and 70 ± 5% relative humidity (RH), were tested in the present study as is summarized in Table 1.

Gloeophyllum sepiarium (Wulf.: Fr.) P. Karst., Strain Number 735 LPC and *Pycnoporus sanguineus* (L.) Murrill, Strain Number 163 LPC, responsible of brown rot and white rot, respectively, frequently used in studies of this type [62–64] were used for the biodeterioration tests. Each strain was axenically cultivated in Petri dishes with a malt-agar medium (composition: 20 g agar and 25 g malt in 1000 mL of distilled water) and incubated for 10 days at 23 ± 2 °C and 70 ± 5% RH. At this stage, the aim was to collect the amount of inoculum required for the tests listed in Table 1.

Table 1. Type, condition and distribution of material used for the biodegradation assays (adapted from EN 113-1:2020 standard).

Timber	Condition	Number of Samples	
		Gloeophyllum sepiarium [1]	*Pycnoporus sanguineus* [2]
Populus euramericana I-214 (TM)	untreated	30	30
	IM1 [3] (0.375 mg/mL)	30	30
	IM2 [3] (0.750 mg/mL)	30	30
Fagus sylvatica (RM)	Reference assay	30	30

[1] brown-rot fungus. [2] white-rot fungus. [3] doses are referred to the concentration of tebuconazole in the impregnation mixture.

To assess the efficacy of the biocidal product in the 2 proposed concentrations, TM and RM specimens were introduced into the culture vessels (2 TM or 2 RM at the same status per vessel) after their sterilization (except treated wood) and determination of their initial dry mass (M_i). After 16 weeks under controlled temperature and humidity (23 ± 2 °C and 70 ± 5% RH) in the culture chamber, the RM samples were extracted from the vessels, conditioned (extraction of the surface mycelium), and oven-dried at 30 ± 2 °C to determine their final dry mass (M_f). Subsequently, degradation was determined by the loss in mass as a percentage of the initial dry mass (ML(%)) according to the following expression:

$$\mathrm{ML}(\%) = \frac{(Mi - Mf)}{Mi} \times 100. \quad (3)$$

Once a ML(%) higher than 20% was detected on a specific reference material (established test reliability), the same procedure was carried out for the TM samples. As was stated above, 240 samples were used, 60 for each type of wood and experimental treatment (TM untreated; TM treated with two different doses of the tebuconazole and RM). Thirty replicates were performed for each strain of xylophagous fungus (see Table 1).

2.7. Evaluation of Tebuconazole Amount Retained into Wood after Impregnation

The indirect quantification of tebuconazole retained in the wood was determined from the difference between the concentration of tebuconazole in the impregnation mixture at the beginning and the end of the impregnation process. The determination of the amount of tebuconazole impregnated in the wood relies on two parts: (i) extraction of tebuconazole from the impregnation mixtures (IM), and mixtures recovered before (IMR_{BV}) and after (IMR_{AV}) the application of the final vacuum (650 mmHg, 15 min), i.e., before and after step vi of the impregnation process, respectively, and (ii) use of gas chromatography with flame ionization detection (GC-FID) analysis for determining the tebuconazole concentration (mg/mL) remaining within the recovered samples, followed by an indirect estimation of the amount of tebuconazole impregnated into the wood. Figure 2 displays a flow chart representing the steps of the impregnation process, and the points in which the different mixtures used for quantification of the tebuconazole amount impregnated into the wood, i.e., IM, IMR_{BV} and IMR_{AV}, are recovered.

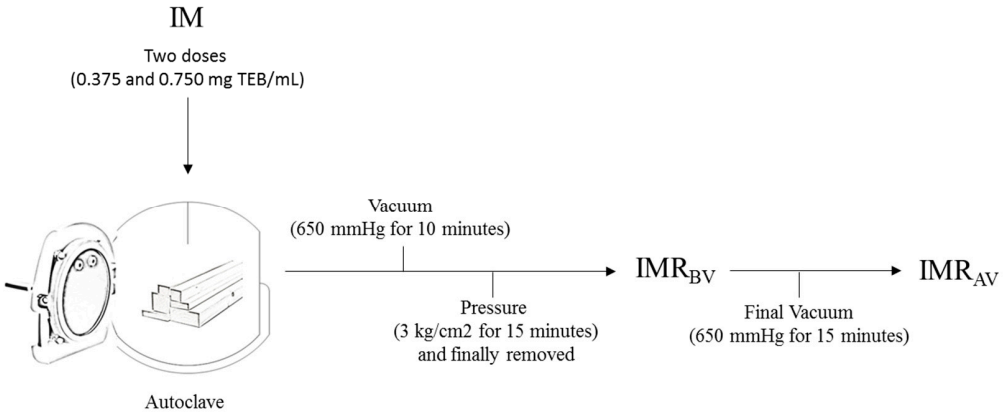

Figure 2. Flow chart representing the different steps of the impregnation process, and the different points in which the mixtures used for the quantification of the tebuconazole amount impregnated into the wood are recovered.

The preparation of samples for GC-FID determination of the tebuconazole concentration in the three recovered mixtures during the impregnation process was done by the dilution of an aliquot (2.5 mL) in 2.5 mL of dichloromethane. The obtained samples were shaken (5 min), and then sonicated (30 min), avoiding that the temperature exceeds 25 °C. Afterwards, the samples were left for stabilization overnight before their injection in a GC-FID instrument (QP2010 Ultra, Shimadzu) fitted with a DB-5MS column (30 m length × 0.25 mm diameter, Agilent Technologies, Santa Clara, CA, USA). This makes possible the determination of the tebuconazole concentration (mg/mL) in the injected samples, with the tebuconazole concentration in the three recovered samples being obtained upon the application of the corresponding dilution factors. The determination of the tebuconazole amount on the recovered samples requires of a calibration curve. This was carried out using solutions in dichloromethane of tebuconazole in the concentration range 0.05–1 mg/mL, with the content of tebuconazole being evaluated from the total area of the peak corresponding to the presence of such molecule. Three replicates of each sample were analyzed, and the tebuconazole concentration in each recovered sample was determined as the average value.

The tebuconazole concentration retained in the wood after the impregnation process, $IMRe^C$ (mg/mL), can be obtained as follows:

$$IMRe^C\left(\frac{mg}{mL}\right) = \frac{\left(IM^C \times IM^V\right) - \left[\left(IMR^C_{BV} \times IMR^V_{BV}\right) + \left(IMR^C_{AV} \times IMR^V_{AV}\right)\right]}{\left(IMRe^V\right)}, \quad (4)$$

with IM, IMR_{AV} and IMR_{BV} being referred again to the impregnation mixture, and the mixtures recovered before and after the application of the final vacuum, respectively. The super-indexes C and V indicates the concentration (mg/mL) and volume (mL) of the different recovered mixtures, respectively. $IMRe^V$ is the volume (mL) of impregnation mixture retained into the wood, which is obtained by difference between the initial impregnated volume and the volumes recovered before and after the application of the final vacuum, i.e., $IMRe^V = IM^V - \left(IMR^V_{BV} + IMR^V_{AV}\right)$. From the value of the concentration of tebuconazole in the mixture retained in the wood (Equation (4)), its volume and the mass of the impregnated wood, the amount of tebuconazole retained per gram of impregnated wood can be estimated. This procedure was performed independently for the two doses used for impregnation.

2.8. Statistical Analysis

The assumptions of independence, normality, and homoscedasticity were checked for all groups with a Kolmogorov-Smirnov, Bartlett's and Cochran's tests using the software

STATGRAPHICS Plus for Windows Version 4.0 (SGWIN P® Corp., The Plains, VA, USA) and Origin Lab 8.0 (OriginLab Corporation, Northampton, MA, USA). The density values were analysed using Student's *t*-test. The mass Loss (log N-transformed) values were analysed using analysis of variance (ANOVA), and the mean values were compared by the Tukey test. In all cases, differences were considered significant when p value < 0.05.

3. Results and Discussion

3.1. Density and sTatability of Aqueous Dispersions of Eugenol Droplets Loaded with Tebuconazole

Table 2 shows the values obtained for the densities of water, solution of poloxamer 407, bare eugenol in water nanoemulsions without tebuconazole and dispersion of tebuconazole carriers at 25 °C (note that nanoemulsions were left for stabilization and homogenization during 24 h before their analysis). The results show that the eugenol in water nanoemulsions containing tebuconazole within the essential oil compound droplets present a density higher than water, which is not surprising considering the higher density of solid tebuconazole (1.25 g/cm^3) and eugenol (1.06 g/cm^3) in relation to water.

Table 2. Composition and density values as were obtained for water, poloxamer 407 solution, eugenol in water nanoemulsion and of dispersion of tebuconazole carriers. Each density value represents the average of 10 independent replicates with their respective standard deviation. Means in same column followed by different letters are significantly different by Student *t*-test for $p < 0.05$.

Sample	Water (wt%)	Poloxamer 407 (wt%)	Eugenol (wt%)	Tebuconazole (wt%)	Density (g/cm^3)
Water	100	0	0	0	1.001 ± 0.006 [a]
Poloxamer 407 solution	82.5	7.5	0	0	1.05 ± 0.01 [b]
Eugenol in water nanoemulsion	88.5	7.5	4	0	1.07 ± 0.02 [bc]
Dispersion of tebuconazole carriers	87	7.5	4	1.5	1.067 ± 0.005 [bc]

In addition to the characterization of the density of the obtained formulations, the development of systems with potential technological application also requires the existence of long-term stability. For this purpose, dynamic light scattering, DLS, experiments were performed for the characterization of the size of the droplets, as their apparent hydrodynamic diameter, d_h^{app}, within which tebuconazole molecules are dispersed. Figure 3 shows the intensity auto-correlation functions obtained for the dispersions of tebuconazole carriers stored during different times (storage time) at 25 °C.

The results show that the dynamic behavior of nanoemulsions loaded with tebuconazole at 25 °C is relatively complex, presenting a clear bimodal character independent of the storage time. Nevertheless, the dynamic behavior and, in particular, the characteristic relaxation times evolves with the storage of the emulsions at 25 °C. Thus, the fast relaxation process, i.e., that appearing with the shortest value of the characteristic time, which is associated with the smallest droplets remains relatively unchanged with the storage at 25 °C, whereas the slow relaxation process, i.e., that appearing at a long characteristic time, increases with the storage time. This may be related to the impact of the storage on the stability of the initial emulsions. A better understanding of this change can be gained in terms of the d_h^{app} distributions obtained from the analysis of the intensity auto-correlation functions (see Figure 3b). The apparent hydrodynamic diameter distributions show the presence of two well-separated populations of droplets, in agreement with the bimodal character of the intensity auto-correlation functions.

Moreover, the decrease of the coherence of the intensity autocorrelation functions to the storage time at 25 °C increases gives an indication of the decrease of the average concentration of scatters in the aqueous environment, i.e., the reduction of the number droplets of eugenol loaded with tebuconazole. This may be explained considering the possible coalescence and/or Ostwald ripening phenomena that results, according to the relative

values of the densities of the different components of the dispersions, which may drive the sedimentation of the biggest droplets at the bottom of the container. This is clear from the apparent hydrodynamic diameter distributions shown in Figure 3b. Initially, the contribution of the two populations is similar, even though considering that the scattered intensity increases with the sixth power of the size, it may be expected a higher concentration of droplets with the smallest size. However, the importance of the contribution corresponding to the biggest droplets decreases with the storage time, whereas the importance of the contribution of the droplets with the smallest size increases. This suggests a partial destabilization of the dispersions of tebuconazole carriers upon storage at 25 °C, which may result in a partial sedimentation of the oil droplets loaded with the tebuconazole. Despite this instability, it is possible to redisperse phase separated stock dispersions upon dilution to prepare homogeneous impregnation mixtures. These impregnation mixtures present a monodisperse character and a size distribution independent on the storage time at 25 °C before their preparation upon dilution. Figure 3c,d show the intensity autocorrelation functions and the resulting apparent hydrodynamic diameter distributions, respectively, for impregnation mixtures containing tebuconazole in concentration 0.375 mg/mL obtained upon dilution of the stock dispersion after storage during different times at 25 °C.

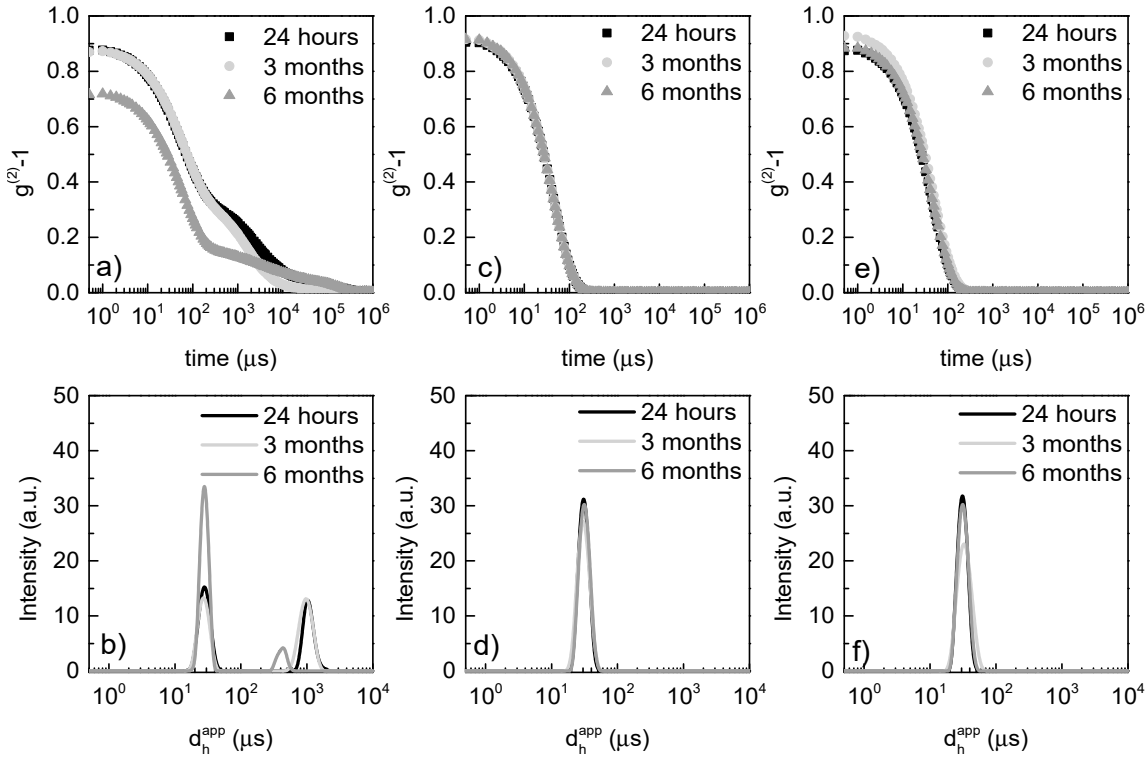

Figure 3. (**a**) Intensity auto-correlation functions for eugenol in water nanoemulsions loaded with tebuconazole (stock dispersion) after different times of storage at 25 °C. (**b**) Intensity-apparent hydrodynamic diameter distributions (obtained from the analysis of intensity auto-correlation functions in panel (**a**)) for eugenol in water nanoemulsions loaded with tebuconazole (stock dispersion) after different times of storage at 25 °C. (**c**) Intensity auto-correlation functions for impregnation mixtures with tebuconazole concentration of 0.375 mg/mL obtained upon dilution a stock dispersion after different times of storage at 25 °C. (**d**) Intensity-apparent hydrodynamic diameter distributions (obtained from the analysis of intensity auto-correlation functions in panel (**c**)) for impregnation mixtures with tebuconazole concentration of 0.375 mg/mL obtained upon dilution a stock dispersion after different times of storage at 25 °C. (**e**) Intensity auto-correlation functions for impregnation mixtures with tebuconazole concentration of 0.750 mg/mL obtained upon dilution of the stock dispersion after different times of storage at 25 °C. (**f**) Intensity-apparent hydrodynamic diameter distributions (obtained from the analysis of intensity auto-correlation functions in panel (**e**)) for impregnation mixtures with tebuconazole concentration of 0.750 mg/mL obtained upon dilution of the stock dispersion after different times of storage at 25 °C.

The results evidence that even though the obtained stock dispersions are not stable systems, undergoing destabilization process during their storage at 25 °C, they can be used for preparing one phase impregnation mixtures formed by droplets with an average apparent hydrodynamic diameter of about 30 nm. These impregnation mixtures were easily obtained only by the addition of water to the concentrated stock dispersions. This suggest that concentrated stock dispersions may be exploited as concentrated emulsions loaded with tebuconazole, which can be diluted for obtaining impregnation mixtures. It should be noted that it is also possible to obtain impregnation mixtures with a higher tebuconazole content (0.750 mg/mL) by twenty times dilution of the stock dispersions. These impregnation mixtures with tebuconazole concentration of 0.750 mg/mL do not present any significant difference in their physico-chemical behavior in relation to those having only 0.375 mg/mL as evidenced the results of DLS experiments shown in Figure 3e,f.

The above discussion has shown that the stock dispersions undergo destabilization process upon their storage during 6 months at 25 °C. For evaluating, the potential impact of the storage conditions on the stability of the stock dispersions, their stability upon storage at 5 °C was also evaluated by DLS. Figure 4a shows the intensity autocorrelation functions and the resulting apparent hydrodynamic diameter, d_h^{app}, distributions for the stock dispersions upon storage during different times at 5 °C.

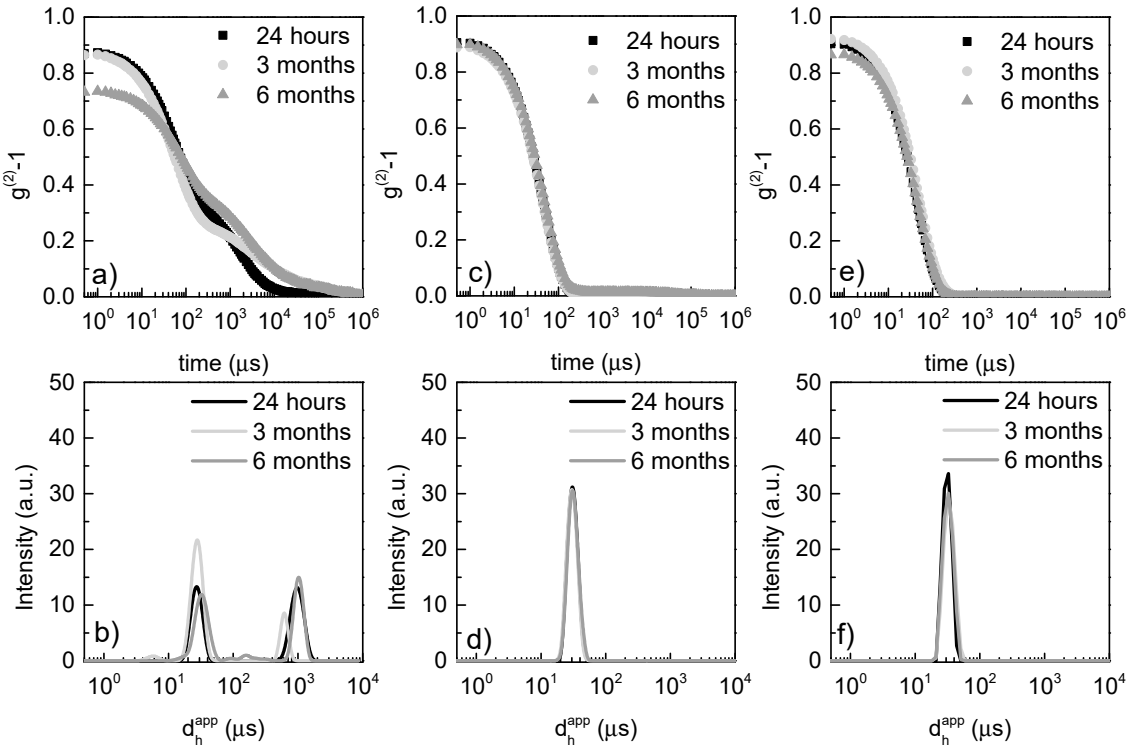

Figure 4. (**a**) Intensity auto-correlation functions for eugenol in water nanoemulsions loaded with tebuconazole (stock dispersion) after different times of storage at 5 °C. (**b**) Intensity-apparent hydrodynamic diameter distributions (obtained from the analysis of intensity auto-correlation functions in panel (**a**)) for eugenol in water nanoemulsions loaded with tebuconazole (stock dispersion) after different times of storage at 5 °C. (**c**) Intensity auto-correlation functions for impregnation mixtures with tebuconazole concentration of 0.375 mg/mL obtained upon dilution a stock dispersion after different times of storage at 5 °C. (**d**) Intensity-apparent hydrodynamic diameter distributions (obtained from the analysis of intensity auto-correlation functions in panel (**c**)) for impregnation mixtures with tebuconazole concentration of 0.375 mg/mL obtained upon dilution a stock dispersion after different times of storage at 5 °C. (**e**) Intensity auto-correlation functions for impregnation mixtures with tebuconazole concentration of 0.750 mg/mL obtained upon dilution of the stock dispersion after different times of storage at 5 °C. (**f**) Intensity-apparent hydrodynamic diameter distributions (obtained from the analysis of intensity auto-correlation functions in panel (**e**)) for impregnation mixtures with tebuconazole concentration of 0.750 mg/mL obtained upon dilution of the stock dispersion after different times of storage at 5 °C.

The stock dispersion after storage at 5 °C (Figure 4b) present a similar behavior to that discussed above for dispersions stored at 25 °C (Figure 3b), existing again a certain reduction of the contain of eugenol droplets as evidenced the reduction of the coherence factor. Therefore, the storage at temperatures in the range 5–25 °C does not appear as a limiting factor for controlling the stability of the formulations. It should be noted that the dilution of stock dispersions stored at 5 °C during different times leads to impregnation mixtures similar to that shown above for impregnation mixtures obtained from dilution of concentrated stock dispersions stored at 25 °C. Figure 4c–f display the DLS results, i.e., intensity autocorrelation functions and the resulting apparent hydrodynamic diameter distributions, for impregnation mixtures obtained by diluting stock dispersions stored at 5 °C. The above discussion has demonstrated that the inclusion of tebuconazole on eugenol in water stabilized with poloxamer 407 results in a certain instability of the formulation upon storage at 5 and 25 °C. However, the phase separated dispersions can be used as concentrated emulsions for preparing impregnation mixtures upon dilution with water.

3.2. Evaluation of the Amount of Tebuconazole Retained into Wood

The retention is considered as the amount of preservative remaining in the wood after finishing the impregnation process, and is expressed in terms of the weight of active ingredient contained in one cubic meter of treated wood (kg/m^3) [65]. In this work, the retention of tebuconazole in wood was evaluated using gas chromatography with flame ionization detection (GC-FID) according to the procedure described in Section 2.8 and expressed as mg of tebuconazole retained per gram of treated wood (mg/g). This quantity of tebuconazole retained per gram of treated wood combined with the value of the wood density (400 kg/cm^3) provides an estimation of the retention in kg/m^3 unit. The first step for the determination of the amount of tebuconazole retained into the wood is to make a calibration curve using solutions of tebuconazole in dichloromethane with concentrations (c_{teb}) in the range 0–1 mg/mL. Figure 5 displays the obtained calibration curve for the solutions of tebuconazole in dichloromethane.

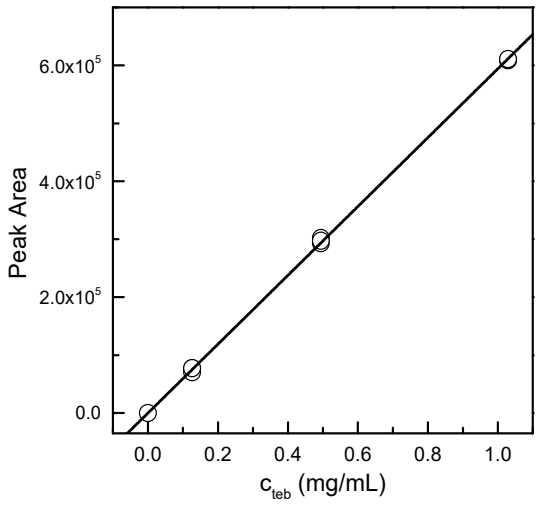

Figure 5. Calibration curve obtained from measurements of tebucanozale solutions in dichloromethane.

The calibration curve shows a good linearity in the tebuconazole concentration range 0–1 mg/mL and can be used for evaluating indirectly the retention of tebuconazole in the wood samples. The application of impregnation mixtures containing two different tebuconazole concentrations (c_{Teb}) was tested (0.375 and 0.75 mg/mL). Before the application of the impregnation mixtures in wood pieces, the real concentration of tebuconazole in the impregnation mixture (IM) was evaluated by GC-FID resulting in concentration values of 0.39 ± 0.02 (IM1) and 0.75 ± 0.02 (IM2), respectively. The accordance between

the determined concentration for the impregnation mixture by GC-FID, and the expected considering that the dispersion of tebuconazole upon dilution of the stock dispersions occurs homogenously confirms the possible use of the eugenol in water emulsions loaded with tebuconazole as a concentrated emulsion for wood impregnation. Furthermore, these results support the use of GC-FID on the determination of tebuconazole concentration in dispersions, allowing for quantification of the amount of tebuconazole impregnated in wood from the amount of tebuconazole remaining in the dispersion obtained after impregnation. Table 3 summarizes the concentrations and volumes of the mixtures recovered after the two stages of the impregnation process (without and with vacuum) and the final retention calculated from the obtained results.

Table 3. Volumes and concentrations of the remaining dispersions after the two stages of the impregnation process, and final retention of tebuconazole upon impregnation. Each value represents the average of two independent replicates with their respective standard deviation.

IM [1]	Wood Mass (g)	c_{Teb} (mg/mL)	IMR_{BV} [2]		IMR_{AV} [3]		Retention (mg/g)
			c_{Teb} (mg/mL)	Volume (mL)	c_{Teb} (mg/mL)	Volume (mL)	
IM1	543.46	0.39 ± 0.02	0.36 ± 0.01	1100	0.25 ± 0.02	105	0.522
IM2	534.61	0.75 ± 0.02	0.71 ± 0.03	1050	0.57 ± 0.04	105	1.041

[1] IM: impregnation mixture. [2] IMR_{BV}: mixture recovered before the application of the final vacuum. [3] IMR_{AV}: mixture recovered after the application of the final vacuum (650 mmHg, 15 min).

The recovered dispersions after the impregnation of wood with 1800 mL of IM1, i.e., IMR_{BV} (mixture recovered before the application of the final vacuum) and IMR_{AV} (mixture recovered after the application of the final vacuum), present concentrations of 0.36 ± 0.01 mg/mL (1100 mL) and 0.25 ± 0.02 mg/mL (105 mL) as were determined using GC-FID, respectively. This results in a retention of 595 mL of the initial impregnation mixture within the wood, which using Equation (4) and considering that the impregnated wood has a mass of 543.46 g leads to a retention of 0.522 mg of tebuconazole per gram of wood, which represents a final retention of about 40% of the initial amount contained in the impregnation mixture.

Following similar approach to the above discussed for t sample IM1, the impregnation of wood with 1800 mL of IM2 was also tested. In this case, the volumes and concentrations of tebuconazole determined by GC-FID on the recovered mixtures before (IMR_{BV}) and after (IMR_{AV}) the application of the final vacuum (650 mmHg, 15 min) were 1050 mL and 0.71 ± 0.03 mg/mL, and 105 mL and 0.57 ± 0.04 mg/mL, respectively. Thus, taking into account that 645 mL of the initial impregnation mixtures were retained by the 534.61 g of wood, and applying equation 4, the amount of tebuconazole impregnated into the wood was 1.041 mg per gram of wood. This leads again to a final retention close to 40% (exactly 41%) of the initial tebuconazole contained in the impregnation mixture.

The estimated retention values in kg/m^3 for the two tebuconazole doses evaluated in this study, 0.375 and 0.750 mg/mL, were 0.2208 and 0.4164, respectively appears in the similar range than those reported in the literature. Liu et al. [66,67] found that the impregnation of wood with dispersions of nanoparticles containing tebuconazole allows reaching a maximum retention of about 0.8 kg tebuconazole/m^3 wood. Furthemore, they developed different polymer nanoparticles of polyvinylpyridine (PVP), copolymers of PVP and styrene (PVP-co-St), and blends of PVP and hyperbranchedpolyesters (HBPs) for loading tebuconazole [68]. These particles present average diameters in the range 100–250 nm and can be used for the impregnation of Southern yellow pine (SYP) wood by conventional vacuum-pressure treatments, resulting in an almost quantitative release of tebuconazole up to reaching a maximum retention in the range 0.4–0.7 kg tebuconazole/m^3 wood, depending on the specific characteristics of the particles.

Wood preservatives products are commonly classified within three different categories: (i) oil-borne (creosote); (ii) water-borne (chromate copper arsenate (CAA); borate;

copper azole, and alkaline cooper quaternary (ACQ)), and (iii) organic solvent-borne. The effectiveness of these product depends largely on their retention into wood, which is evaluated by the American Wood Protection Association standard (AWPA standard) according to the minimal amount of retained preservative within the wood under specific service conditions of use of the material [69]. In particular, the UCB3 category defines that for wood exposed to severe weather events, including prolonged wetting (exterior constructions above uncoated ground or with poor water run-off), the retention values should be in the ranges 0.25–0.30 kg/m^3 for arsenical preservatives, 0.07–0.206 kg/m^3 for non-arsenical copper preservatives and 0.013–0.019 kg/m^3 for other preservatives, including mixtures containing propiconazole and tebuconazole [69].

It is clear that the impregnation capacity of the here prepared formulations appears more than one order of magnitude higher than the minimal value required for formulations containing tebuconazole according to the above-mentioned standard, and hence it can be assumed that formulations based on eugenol in water nanoemulsions loaded with tebuconazole emerge as a very promising tool for preparing dispersible concentrates aimed to wood protection. It should be noted that even though the amount of tebuconazole included in the formulations may be associated with high production costs, there are some aspects that are important to consider from an economic and ecological perspectives. On one side, the formulations are aqueous based products (more than 80 wt% of the formulation is water) that can be prepared in situ without any complex instrumentation. This reduces significantly the amounts of products that must be transported from the warehouses to the application points, which reduces transportation costs and contributes to minimize the carbon dioxide footprint. On the other side, these formulations are mostly composed for renewable compounds (water and eugenol are more than the 90 wt% of the total weight of the formulation), which allows the production of more sustainable formulations in relation to traditional formulations. Therefore, the combination of the high retention into the wood with the reduction of the economic and ecological costs associated with the production of the formulations suggests that eugenol in water nanoemulsions loaded with tebuconazole can be used as alternative technology for replacing the products commonly used for wood protection [24].

Moreover, the results evidence that independently of the content of tebuconazole in the initial dispersion, the degree of retention is about 40% of the initial content of tebuconazole contained in the impregnation mixture. This indicates the absence of physical saturation of the wood within the range of tebuconazole concentration evaluated because the increase of the tebuconazole amount contained in the impregnation mixture dispersion leads to an increase of the retained amount for the same factor, i.e., when the concentration of the impregnation mixtures increased by a factor two, the amount of tebuconazole retained in the wood also presents the same increase. This combined with the retention level are essential parameters to define the category of treated wood under specific operation conditions and make possible that the stock solutions can be used for preparing more diluted impregnation mixtures for adapting retention level to that what are marked by the international standards.

3.3. Evaluation of the Protection of Impregnated Wood against White- and Brown-Rot Decay Caused by Fungus

Once the effectiveness of the wood impregnation with tebuconazole has been evaluated, the evaluation of the achieved degree of protection requires an examination of their resistance against the attack of xylophagus fungi. This can be done in terms of the mass loss percentage (ML%) upon exposure to different strains of fungi. Table 4 displays the average percentage of mass loss (ML%) for poplar (untreated and treated) and beech (reference material) woods upon exposure to *Gloeophyllum sepiarium* and *Pycnoporus sanguineus* fungi.

Table 4. Effect of treatments on mass loss for *Populus canadensis* I-214 wood-blocks exposed to *Gloeophyllum sepiarium* and *Pycnoporus sanguineus*. Notice that two different doses of tebuconazole were applied to the different woods: IM1 (0.375 mg/mL) and IM2 (0.750 mg/mL).

Xylophagous Fungi	Timber	Condition	ML (%) [3]
Pycnoporus sanguineus [1] (Strain 163 LPC)	*P. euramericana* I-214	untreated	30 ± 16
		IM1	2 ± 1
		IM2	2 ± 1
	Fagus sylvatica	Reference material	32 ± 13
Gloeophyllum sepiarium [2] (Strain 735 LPC)	*P. euramericana* I-214	untreated	22 ± 7
		IM1	2 ± 1
		IM2	1 ± 1
	Fagus sylvatica	Reference material	26 ± 8

[1] White-rot fungus strain. [2] Brown-rot fungus strain. [3] ML (%): percentage of mass loss (%) (notice that each value represents the average of 30 independent replicates with their respective standard deviation).

The largest ML (%) was for untreated woods, both *Populus euramericana I-214* and *Fagus sylvatica*, and in particular for the material affected by white rot, in which the mass losses assume values about 30%. This is in agreement with the results reported by Spavento et al. [70]. They determined the susceptibility of *Populus euramericana I-214* to xylophagous basidiomycete fungi *(Coriolus versicolor and Coniophora puteana),* and concluded that such type of poplar wood should be considered as non-durable wood according to the standard EN 350:2016, which defines testing procedures for evaluating the durability to biological agents of wood and wood-based materials and provides a classification of the durability in different categories [71]. Furthermore, it should be noted that the mass losses found for untreated woods present high standard deviation values, which may be explained assuming the heterogeneity expected for a natural product.

On the other hand, poplar wood impregnated with the formulations containing tebuconazole presents a very small mass loss (in the range 0–2%), independently of the dose used for the treatment. Therefore, the treated samples, independently of the tebuconazole dose used for the treatment, increase the resistance of wood against the degradation induced by the exposure to xylophagous fungi. This increase in the resistance of the wood upon treatment validates the formulations for wood protection. The above discussed increase of the wood durability upon the treatment with the tebuconazole formulation is similar to that obtained for *Populus nigra* wood treated with chromated copper arsenate (CCA), which is considered a very effective preservative, by vacuum-pressure [61]. However, considering the current restrictions associated with the use of CCA, the obtained results offer a very interesting opportunity for substituting CCA for a more eco-sustainable formulations for wood protection [72,73].

As mentioned above, Liu et al. [66–68] studied the action of nanoparticles loaded with tebuconazole as a preservatives for Southern yellow pine (SYP) and birch wood against white- and brown-rot fungi, and found that such nanoparticles provide a very good resistance against fungal attack on treated wood, even at very low levels of retention (0.1–0.8 kg tebuconazole/m^3 wood). In all cases, the Southerm yellow pine mass loss was less than <5% after 55 days of exposure to *Gloeophyllum trabeum* (responsible for brown rot) when the retention into wood was only 0.4 kg tebuconazole/m^3 wood. Moreover, for birch wood, 0.8 kg of tebuconazole/m^3 of wood were enough to reduce the mass loss below 5% of the initial mass after 55 days of exposure to *Trametes versicolor* (responsible for brown rot). According to Liu et al. [68], mass losses below 5% may be considered negligible in relation to the resistance against fungal degradation, and this level was reached for SYP wood with a tebuconazole load of about 0.2 kg tebuconazole/m^3 wood, which is well below the usual target loading for tebuconazole.

The evidenced resistance to the decay determined for impregnated poplar wood upon exposure to white and brown rot fungi provides additional insights on the efficacy of the designed formulations containing a triazole (tebuonazole) for wood preservation [6]. The treatment of the wood with the experimental formulations may counteract the natural tendency to biodegradation of this material, which may help on the reutilization of the treated piece once its service life of a specific structure is over.

It should be noted that a successful protection of the wood using the studied formulations should consider additional aspects. First, the chemical heterogeneity and the porous structure of the wood which can result in an asymmetric penetration of the preservative molecules [74,75]. Therefore, the optimization of the formulations requires a careful examination of the ability of the preservative to flow into wood capillaries, which is mainly controlled by the viscosity of the formulations and their polar/apolar character. The latter governs the adhesion of the formulations to the wood [76]. Furthermore, the wood pores has an average diameter in the range 400–600 nm [77], which limits the use of aqueous formulation containing micron sized particles [78–80]. However, the size of droplets of the impregnation mixtures developed in this work (around 30 nm) may favor a fast penetration of the tebuconazole through the wood pores excluding the water, which makes of the developed formulations suitable candidates for an optimal impregnation of the wood. Furthermore, the evaporation of the water occurring during the impregnation process may contribute to the penetration of the oil droplets containing tebuconazole as a result of the destabilization process occurring in the formulation as the water evaporates. This may lead to the sedimentation of an oil layer on the surface of the wood, which avoid the penetration of the water during the impregnation process.

4. Conclusions

This study has demonstrated the possible design of formulations containing tebucanozole as concentrated emulsions using eugenol in water nanoemulsions stabilized by Poloxamer 407. These nanoemulsions offers a suitable platform for an efficient encapsulation and dispersion of a poorly water-soluble active molecule (tebuconazole) in water. It is true that the stock dispersions can undergo destabilization process during their storage, which leads to the reduction of the active ingredient concentration dispersed within the nanoemulsion. However, the active ingredient can be efficiently redispersed upon dilution to obtain impregnation dispersions with a relatively monodisperse character. The impregnation of poplar wood with the diluted mixtures (impregnation mixtures) leads to a high degree of retention of the tebuconazole (around 40% of the total content of the formulation), which enhances significantly the resistance of the wood against xylophagous fungi attack. Therefore, the here developed formulations open a very interesting avenue on the design of new eco-sustainable products for wood protection, minimizing the use of surfactant and organic solvents commonly used for ensuring the dispersion of poorly water-soluble molecules, and hence the formulations containing tebuconazole may appear as an alternative to the use of the CCA as well as to the use of oil-soluble preservatives, resulting in formulations with a very low environmental impact. Furthermore, the use of aqueous formulations would contribute to reduce the risk of fire during the handling and transport. Furthermore, the simplicity of the methodology used for the preparation of the formulations appears as an additional advantage for the developed formulations, facilitating an in-situ preparation of the formulation, which reduces the costs associated transport and the CO_2 footprint. It should be noted that considering the low-moderate solubility of tebuconazole in water, which makes tebuconazole susceptible of washing (leaching), it is necessary a further evaluation of durability of the impregnated wood with the designed formulation after leaching. Thus, it would be possible to obtain a more realistic perspective of the effectiveness of the formulation under real environmental exposure conditions.

Author Contributions: Conceptualization, A.L. and M.M.; methodology, A.L., G.S. and R.C.; software, A.L. and E.G.; validation, A.L., M.M.; G.K. and R.C.; formal analysis, A.L. and E.G.; investigation, A.L., M.M., G.S., G.K., R.C., R.G.R. and E.G.; resources, A.L., M.M. and R.C.; data curation, A.L. and E.G.; writing—original draft preparation, A.L., G.S. and E.G.; writing—review and editing, A.L., M.M., G.S., G.K., R.C., R.G.R. and E.G.; visualization, A.L. and E.G.; supervision, A.L. and M.M.; project administration, A.L. and E.G.; funding acquisition, A.L. and E.G. All authors have read and agreed to the published version of the manuscript.

Funding: A.L. and G.S. acknowledge to Facultad de Ciencias Agrarias y Forestales for the scholarship granted to carry out a final thesis under the Programa de Participación Estudiantil (Res N° 571/16). The part performed in Spain was funded by MICINN (grant PID2019-106557GB-C21), by Banco Santander-Universidad Complutense (grant PR87/19-22513) and by E.U. on the framework of the European Innovative Training Network–Marie Sklodowska–Curie Action Nano Paint (grant agreement 955612). The part performed in Argentina was funded under grant project titled "Tecnologías aplicadas en madera de álamos (*Populus* spp) implantados en sistemas silvopastoriles de Río Negro y Neuquén". (Código 11 A 291. Programa de Incentivos del Ministerio de Educación de La Nación. 2016–2019).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No data available.

Acknowledgments: Authors are grateful to the Research Institute of Theoretical and Applied Physical Chemistry (INIFTA/UNLP-CONICET, La Plata, Argentina) for the use of their Dynamic Light Scattering facilities. Authors also acknowledge to the Laboratorio de Investigaciones en Maderas (Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina) their help on the cutting and conditioning process of the wood for the assays and to Química Bosques, S.A. for providing the facilities for the impregnation process. AL, MM and GS would like to thank R.C., from Empresa Química Bosques S.A (Argentina) for facilitating the pilot plant where the impregnation tests were carried out.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Ramage, M.H.; Burridge, H.; Busse-Wicher, M.; Fereday, G.; Reynolds, T.; Shah, D.U.; Wu, G.; Yu, L.; Fleming, P.; Densley-Tingley, D.; et al. The wood from the trees: The use of timber in construction. Renew. *Sust. Energ. Rev.* **2017**, *68*, 333–359. [CrossRef]
2. Chang, S.-T.; Hon, D.N.-S.; Feist, W.C. Photodegradation and photoprotection of wood surfaces. *Wood Fiber Sci.* **1982**, *14*, 104–117.
3. Highley, T.L. *Biochemical Aspects of White-Rot and Brown-Rot Decay*; International Research Group on Wood Preservation: Stockholm, Sweden, 1987.
4. Zabel, R.; Morrell, J. *Wood Microbiology*; Academic Press: Waltham, MA, USA, 2020.
5. Schmidt, O. *Wood and Tree Decay. Biology, Damage, Protection and Use*; Springer: Berlin, Germany, 2006.
6. Lebow, S.T. Wood Preservation. In *Wood Handbook. Wood as an Engineering Material*; Department of Agriculture, Forest Service, Forest Products Laboratory: Madison, WI, USA, 2010; pp. 15.11–15.28.
7. Thakur, V.K.; Thakur, M.K. Recent Advances in Graft Copolymerization and Applications of Chitosan: A Review. *ACS Sustain. Chem. Eng.* **2014**, *2*, 2637–2652. [CrossRef]
8. Casado-Sanz, M.; Silva-Castro, I.; Ponce-Herrero, L.; Martín-Ramos, P.; Martín-Gil, J.; Acuña-Rello, L. White-Rot Fungi Control on Populus spp. Wood by Pressure Treatments with Silver Nanoparticles, Chitosan Oligomers and Propolis. *Forests* **2019**, *10*, 885. [CrossRef]
9. Silva-Castro, I.; Casado-Sanz, M.; Alonso-Cortés, A.L.; Martín-Ramos, P.; Martín-Gil, J.; Acuña-Rello, L. Chitosan-Based Coatings to Prevent the Decay of Populus spp. Wood Caused by Trametes Versicolor. *Coatings* **2018**, *8*, 415. [CrossRef]
10. Woźniak, M.; Kwaśniewska-Sip, P.; Waśkiewicz, A.; Cofta, G.; Ratajczak, I. The Possibility of Propolis Extract Application in Wood Protection. *Forests* **2020**, *11*, 465. [CrossRef]
11. Torlak, E.; Sert, D. Antibacterial effectiveness of chitosan–propolis coated polypropylene films against foodborne pathogens. *Int. J. Biol. Macromol.* **2013**, *60*, 52–55. [CrossRef]
12. Quiroga, E.; Sampietro, D.; Soberón, J.; Sgariglia, M.; Vattuone, M. Propolis from the northwest of Argentina as a source of antifungal principles. *J. Appl. Microbiol.* **2006**, *101*, 103–110. [CrossRef]

13. Dong, H.; Bahmani, M.; Rahimi, S.; Humar, M. Influence of Copper and Biopolymer/Saqez Resin on the Properties of Poplar Wood. *Forests* **2020**, *11*, 667. [CrossRef]
14. Dorau, B.; Arango, R.; III, F.G. An Investigation into the Potential of Ionic Silver as a Wood Preservative. In Proceedings of the Woodframe Housing Durability and Disaster Issues Conference, Las Vegas, NV, USA, 4–6 October 2004.
15. Kartal, S.N.; Green, F.; Clausen, C.A. Do the unique properties of nanometals affect leachability or efficacy against fungi and termites? *Int. Biodeterior. Biodegrad.* **2009**, *63*, 490–495. [CrossRef]
16. Kim, S.W.; Jung, J.H.; Lamsal, K.; Kim, Y.S.; Min, J.S.; Lee, Y.S. Antifungal Effects of Silver Nanoparticles (AgNPs) against Various Plant Pathogenic Fungi. *Mycobiology* **2012**, *40*, 53–58. [CrossRef]
17. Clausen, C.; Yang, V.; Arango, R.; Green III, F. Feasibility of Nanozinc Oxide as a Wood Preservative. In Proceedings of the One Hundred Fifth Annual Meeting of the American Wood Protection Association, San Antonio, TX, USA, 19–21 April 2009.
18. Matsunaga, H.; Kiguchi, M.; Evans, P.D. Microdistribution of copper-carbonate and iron oxide nanoparticles in treated wood. *J. Nanoparticle Res.* **2009**, *11*, 1087–1098. [CrossRef]
19. Nair, S.; Pandey, K.K.; Giridhar, B.; Vijayalakshmi, G. Decay resistance of rubberwood (*Hevea brasiliensis*) impregnated with ZnO and CuO nanoparticles dispersed in propylene glycol. *Int. Biodeterior. Biodegrad.* **2017**, *122*, 100–106. [CrossRef]
20. Marzbani, P.; Mohammadnia-Afrouzi, Y. Investigation on Leaching and Decay Resistance of Wood Treated with Nano- Titanium Dioxide. *Adv. Environ. Biol.* **2014**, *8*, 974–978.
21. Pánek, M.; Reinprecht, L.; Hulla, M. Ten Essential Oils for Beech Wood Protection—Efficacy Against Wood-destroying Fungi and Moulds, and Effect on Wood Discoloration. *BioResources* **2014**, *9*, 5588–5603. [CrossRef]
22. de Medeiros, F.C.; Gouveia, F.N.; Bizzo, H.R.; Vieira, R.F.; Del Menezzi, C.H. Fungicidal activity of essential oils from Brazilian Cerrado species against wood decay fungi. *Int. Biodeterior. Biodegrad.* **2016**, *114*, 87–93. [CrossRef]
23. Bahmani, M.; Schmidt, O. Plant essential oils for environment-friendly protection of wood objects against fungi. *Maderas Cienc. Tecnol.* **2018**, *20*, 325–332. [CrossRef]
24. Singh, T.; Singh, A.P. A review on natural products as wood protectant. *Wood Sci. Technol.* **2012**, *46*, 851–870. [CrossRef]
25. Caputo, L.; Nazzaro, F.; Souza, L.F.; Aliberti, L.; De Martino, L.; Fratianni, F.; Coppola, R.; De Feo, V. Laurus nobilis: Composition of Essential Oil and Its Biological Activities. *Molecules* **2017**, *22*, 930. [CrossRef]
26. Chittenden, C.; Singh, T. Antifungal activity of essential oils against wood degrading fungi and their applications as wood preservatives. *Int. Wood Prod. J.* **2011**, *2*, 44–48. [CrossRef]
27. Teaca, C.A.; Roşu, D.; Mustaţă, F.; Rusu, T.; Roşu, L.; Roşca, I.; Varganici, C.-D. Natural Bio-Based Products for Wood Coating and Protection against Degradation: A Review. *BioResources* **2019**, *14*, 4873–4902. [CrossRef]
28. Broda, M. Natural Compounds for Wood Protection against Fungi—A Review. *Molecules* **2020**, *25*, 3538. [CrossRef]
29. Loughman, R.; Thomas, G. Fungicide and cultivar control of Septoria diseases of wheat. *Crop. Prot.* **1992**, *11*, 349–354. [CrossRef]
30. Cabras, P.; Angioni, A.; Garau, V.L.; Melis, M.; Pirisi, F.M.; Minelli, E.V.; Cabitza, F.; Cubeddu, M. Fate of Some New Fungicides (Cyprodinil, Fludioxonil, Pyrimethanil, and Tebuconazole) from Vine to Wine. *J. Agric. Food Chem.* **1997**, *45*, 2708–2710. [CrossRef]
31. Buschaus, H.U.; Valcke, A.R. Triazoles: Synergism between Propiconazole and Tebuconazole. In Proceedings of the International Research Group on Wood Preservation Conference, Helsingör, Denmark, 11–16 June 1995.
32. Woo, C.; Daniels, B.; Stirling, R.; Morris, P. Tebuconazole and propiconazole tolerance and possible degradation by Basidiomycetes: A wood-based bioassay. *Int. Biodeterior. Biodegrad.* **2010**, *64*, 403–408. [CrossRef]
33. Joseph-Horne, T.; Hollomon, D.; Loeffler, R.; Kelly, S. Altered P450 activity associated with direct selection for fungal azole resistance. *FEBS Lett.* **1995**, *374*, 174–178. [CrossRef]
34. Reinprecht, L. *Fungicides for Wood Protection—World Viewpoint and Evaluation/Testing in Slovakia*; IntechOpen: Rijeka, Croatia, 2010; pp. 95–122.
35. Eaton, R.A.; Hale, M.D.C. *Wood: Decay, Pests and Protection*; Springer: Berlin, Germany, 1993.
36. Lewis, K.A.; Tzilivakis, J.; Warner, D.J.; Green, A. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess. Int. J.* **2016**, *22*, 1050–1064. [CrossRef]
37. Fishel, F.M. *What Are Inert Ingredients?* Institute of Food and Agricultural Sciences, University of Florida: Gainesville, FL, USA, 2011.
38. Fishel, F.M. *Pesticide Formulations*; Institute of Food and Agricultural Sciences, University of Florida: Gainesville, FL, USA, 2010.
39. *Directive No. 98/8/EC Concerning the Placing of Biocidal Products on the Market*; European Commission: Brussels, Belgium, 1998.
40. Palanivel, V.; Manavalan, R.; Valliappan, K. Microencapsulation: A vital technique in novel drug delivery system. *J. Pharm. Sci. Res.* **2009**, *1*, 26–35.
41. Katiyar, B.; Katiyar, S.; Mishra, P.; Sailaja, D. Microemulsions: A novel drug carrier system. *Int. J. Pharm. Sci. Rev. Res.* **2013**, *20*, 138–148.
42. Perry, S.L.; McClements, D.J. Recent Advances in Encapsulation, Protection, and Oral Delivery of Bioactive Proteins and Peptides using Colloidal Systems. *Molecules* **2020**, *25*, 1161. [CrossRef] [PubMed]
43. Singh, N.; Joshi, A.; Toor, A.P.; Verma, G. Drug delivery: Advancements and challenges. In *Nanostructures for Drug Delivery*; Andronescu, E., Grumezescu, A.M., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 865–886. [CrossRef]
44. Lucia, A.; Guzmán, E. Emulsions containing essential oils, their components or volatile semiochemicals as promising tools for insect pest and pathogen management. *Adv. Colloid Interface Sci.* **2020**, *287*, 102330. [CrossRef] [PubMed]

45. Li, Y.; Zheng, J.; Xiao, H.; McClements, D.J. Nanoemulsion-based delivery systems for poorly water-soluble bioactive compounds: Influence of formulation parameters on polymethoxyflavone crystallization. *Food Hydrocoll.* **2012**, *27*, 517–528. [CrossRef]
46. Harimurti, N.; Nasikin, M.; Mulia, K. Water-in-Oil-in-Water Nanoemulsions Containing Temulawak (*Curcuma xanthorriza* Roxb) and Red Dragon Fruit (*Hylocereus polyrhizus*) Extracts. *Molecules* **2021**, *26*, 196. [CrossRef]
47. Lucia, A.; Guzmán, E.; Rubio, R.G.; Ortega, F. Enhanced solubilization of an insect juvenile hormone (JH) mimetic (piryproxyfen) using eugenol in water nanoemulsions stabilized by a triblock copolymer of poly(ethylenglycol) and poly(propilenglycol). *Colloids Surfaces A Physicochem. Eng. Asp.* **2020**, *606*, 125513. [CrossRef]
48. Fernández-Peña, L.; Gutiérrez-Muro, S.; Guzmán, E.; Lucia, A.; Ortega, F.; Rubio, R.G. Oil-In-Water Microemulsions for Thymol Solubilization. *Colloids Interfaces* **2019**, *3*, 64. [CrossRef]
49. Hosseini, S.F.; Ramezanzade, L.; McClements, D.J. Recent advances in nanoencapsulation of hydrophobic marine bioactives: Bioavailability, safety, and sensory attributes of nano-fortified functional foods. *Trends Food Sci. Technol.* **2021**, *109*, 322–339. [CrossRef]
50. Lucia, A.; Toloza, A.C.; Guzman, E.; Ortega, F.; Rubio, R.G. Novel polymeric micelles for insect pest control: Encapsulation of essential oil monoterpenes inside a triblock copolymer shell for head lice control. *PeerJ* **2017**, *5*, e3171. [CrossRef]
51. Sánchez-Arribas, N.; Guzmán, E.; Lucia, A.; Toloza, A.C.; Velarde, M.G.; Ortega, F.; Rubio, R.G. Environmentally friendly platforms for encapsulation of an essential oil: Fabrication, characterization and application in pests control. *Colloids Surfaces A Physicochem. Eng. Asp.* **2018**, *555*, 473–481. [CrossRef]
52. Lucia, A.; Girard, C.; Fanucce, M.; Coviella, C.; Rubio, R.G.; Ortega, F.; Guzmán, E. Development of an Environmentally Friendly Larvicidal Formulation Based on Essential Oil Compound Blend to Control Aedes aegypti Larvae: Correlations between Physicochemical Properties and Insecticidal Activity. *ACS Sustain. Chem. Eng.* **2020**, *8*, 10995–11006. [CrossRef]
53. Díaz-Blancas, V.; Medina, D.I.; Padilla-Ortega, E.; Bortolini-Zavala, R.; Olvera-Romero, M.; Luna-Bárcenas, G. Nanoemulsion Formulations of Fungicide Tebuconazole for Agricultural Applications. *Molecules* **2016**, *21*, 1271. [CrossRef]
54. Chin, C.-P.; Lan, C.-W.; Wu, H.-S. Application of biodiesel as carrier for insecticide emulsifiable concentrate formulation. *J. Taiwan Inst. Chem. Eng.* **2012**, *43*, 578–584. [CrossRef]
55. Mustafa, I.F.; Hussein, M.Z. Synthesis and Technology of Nanoemulsion-Based Pesticide Formulation. *Nanomaterials* **2020**, *10*, 1608. [CrossRef] [PubMed]
56. Kah, M.; Hofmann, T. Nanopesticide research: Current trends and future priorities. *Environ. Int.* **2014**, *63*, 224–235. [CrossRef] [PubMed]
57. Chhipa, H. Nanopesticide: Current Status and Future Possibilities. *Agric. Res. Technol. Open Access J.* **2017**, *5*, 1–4. [CrossRef]
58. Feng, J.; Zhang, Q.; Liu, Q.; Zhu, Z.; McClements, D.J.; Jafari, S.M. Application of Nanoemulsions in Formulation of Pesticides. In *Nanoemulsions*; Jafari, S.M., McClements, D.J., Eds.; Academic Press: Cambridge, MA, USA, 2018; pp. 379–413. [CrossRef]
59. Konopka, A.; Barański, J.; Orłowski, K.; Szymanowski, K. The Effect of Full-Cell Impregnation of Pine Wood (*Pinus sylvestris* L.) on Changes in Electrical Resistance and on the Accuracy of Moisture Content Measurement Using Resistance Meters. *BioResources* **2018**, *13*, 1360–1371. [CrossRef]
60. *EN 113-1:2020: Durability of Wood and Wood-Based Products—Test Method Against Wood Destroying Basidiomycetes—Part 1: Assessment of Biocidal Efficacy of Wood Preservatives*; European Comittee for Standarization: Brussels, Belgium, 2020.
61. Diaz, B.; Murace, M.; Peri, P.; Keil, G.; Luna, L.; Otaño, M.Y. Natural and preservative-treated durability of Populus nigra cv Italica timber grown in Santa Cruz Province, Argentina. *Int. Biodeterior. Biodegrad.* **2003**, *52*, 43–47. [CrossRef]
62. Bernardi, A.C.; Popoff, O. Durability of Pinus elliottii Wood Impregnated with Quebracho Colorado (Schinopsis balansae) Bio-Protectives Extracts and CCA. *Maderas Cienc. Tecnol.* **2009**, *11*, 107–115. [CrossRef]
63. Gao, J.; Kim, J.S.; Daniel, G. Effect of thermal modification on the micromorphology of decay of hardwoods and softwoods by the white rot fungus *Pycnoporus sanguineus*. *Holzforschung* **2018**, *72*, 797–811. [CrossRef]
64. Kim, J.S.; Gao, J.; Daniel, G. Cytochemical and immunocytochemical characterization of wood decayed by the white rot fungus *Pycnoporus sanguineus* I. preferential lignin degradation prior to hemicelluloses in Norway spruce wood. *Int. Biodeterior. Biodegrad.* **2015**, *105*, 30–40. [CrossRef]
65. AWPA. *Book of Standards*; American Wood Protection Association: Birmingham, AL, USA, 2021.
66. Liu, Y.; Laks, P.; Heiden, P. Controlled release of biocides in solid wood. II. Efficacy against *Trametes versicolor* and *Gloeophyllum trabeum* wood decay fungi. *J. Appl. Polym. Sci.* **2002**, *86*, 608–614. [CrossRef]
67. Liu, Y.; Yan, L.; Heiden, P.; Laks, P. Use of nanoparticles for controlled release of biocides in solid wood. *J. Appl. Polym. Sci.* **2001**, *79*, 458–465. [CrossRef]
68. Liu, Y.; Laks, P.; Heiden, P. Controlled release of biocides in solid wood. I. Efficacy against brown rot wood decay fungus (*Gloeophyllum trabeum*). *J. Appl. Polym. Sci.* **2002**, *86*, 596–607. [CrossRef]
69. Smith, S.T. Water-Borne Wood Preservation and End-of-Life Removal History and Projection. *Engineering* **2020**, *12*, 117–139. [CrossRef]
70. Spavento, E.; Murace, M.; Acuña, L.; Monteoliva, S.E.; Troya, M.T. Susceptibility of *Populus* x *euramericana* 'I-214' of Spanish origin to xylophagous attacks: Durability tests for its possible inclusion in European standard. *For. Syst.* **2019**, *28*, e008. [CrossRef]
71. *EN 350:2016: Durability of Wood and Wood-Based Products—Testing and Classification of the Durability to Biological Agents of Wood and Wood-Based Materials*; European Comittee for Standarization: Brussels, Belgium, 2016.

72. Choi, Y.-S.; Kim, G.-H.; Lim, Y.W.; Kim, S.H.; Imamura, Y.; Yoshimura, T.; Kim, J.-J. Characterization of a strong CCA-treated wood degrader, unknown Crustoderma species. *Antonie Leeuwenhoek* **2009**, *95*, 285–293. [CrossRef]
73. Jennings, P.; Breithaupt, J.; Bays, D.; Dole, T.; Chen, J.; Rury, K.; Luminello, T.; Kyprianou, R.; Ross, P. *Chromated Arsenical Final Work Plan*; EPA-HQ-OPP-2015-0349; Environmental Protection Agency of United States of America: Washington, DC, USA, 2016.
74. Siau, J.F. *Wood-Influence of Moisture on Physical Properties*; Department of Wood Science and Forest Products, Virginia Polytechnic Institute and State University: Blacksburg, VA, USA, 1995.
75. Marcovich, N.; Reboredo, M.; Aranguren, M.I. Moisture diffusion in polyester-woodflour composites. *Polymer* **1999**, *40*, 7313–7320. [CrossRef]
76. Meijer, M.d. Interactions Between Wood and Coatings with Low Organic Solvent Content. Ph.D. Thesis, Agricultural University of Wageningen, Wageningen, The Netherlands, 1999.
77. Bossert, D.; Geers, C.; Placencia Peña, M.I.; Volkmer, T.; Rothen-Rutishauser, B.; Petri-Fink, A. Size and Surface Charge Dependent Impregnation of Nanoparticles in Soft- and Hardwood. *Chemistry* **2020**, *2*, 23. [CrossRef]
78. Civardi, C.; Van den Bulcke, J.; Schubert, M.; Michel, E.; Butron, M.I.; Boone, M.N.; Dierick, M.; Van Acker, J.; Wick, P.; Schwarze, F.W.M.R. Penetration and Effectiveness of Micronized Copper in Refractory Wood Species. *PLoS ONE* **2016**, *11*, e0163124. [CrossRef]
79. Freeman, M.H.; McIntyre, C. A comprehensive review of copper-based wood preservatives with a focus on new micronized or dispersed copper systems. *For. Prod. J.* **2008**, *58*, 6–27.
80. Clar, J.; Platten, W.E.; Baumann, E.J.; Remsen, A.; Harmon, S.M.; Bennett-Stamper, C.L.; Thomas, T.A.; Luxton, T.P. Dermal transfer and environmental release of CeO_2 nanoparticles used as UV inhibitors on outdoor surfaces: Implications for human and environmental health. *Sci. Total. Environ.* **2018**, *613-614*, 714–723. [CrossRef] [PubMed]