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# Optimising Incorporation of Tebuconazole via Chitosan Polymeric Nanoparticles for Semantan Bamboo Preservation

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Tebuconazole performs well as a fungicide but poor water solubility requires alternative delivery methods in vacuum pressure treatments. This can be mitigated by incorporating tebuconazole into chitosan nanoparticles to improve water solubility and delivery. Tebuconazole-loaded chitosan nanoparticle was successfully synthesised *via* nanoprecipitation. Semantan bamboo (*Gigantochloa scortechinii*) samples were treated with the aqueous suspension of the nanoparticles *via* vacuum pressure treatment at 100 psi and 120 min. The bamboo sample was found to possess a good treatability at 250.2 ± 8.87 $Lm^{-3}$ and high chemical retention of 5.55 ± 0.51 $kgm^{-3}$. The tebuconazole-loaded chitosan nanoparticles significantly reduced the percentage weight loss of untreated bamboo from the anti-fungi test, from 22.34 ± 7.97 % to 0.092 ± 0.18 %. The results from this study have shown that the chitosan polymetric nanoparticles can behave as carriers to introduce the tebuconazole into bamboo using vacuum pressure treatment, providing an outstanding resistance against white rot fungi (*Trametes versicolor*).

**Keywords:** Tebuconazole-loaded chitosan nanoparticle; chitosan polymer; nanoprecipitation; bamboo treatment; hydrodynamic particle size; loading efficiency

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Bamboo is one of the fastest growing plants abundant in Malaysia and has attractive attributes such as low materials cost, good mechanical properties and high tensile strength, serving as a valuable resource for furniture, construction and scaffolding purposes. In 2020 it was recorded that Malaysian bamboo exports totalled RM8.28 million. Semantan bamboo is readily available commercially and is widely cultivated throughout Malaysia. Bamboo has rich fibre compositions, but high starch content makes bamboo susceptible to fungi and insect attacks, affecting components such as cellulose and lignin in the structure. To protect the bamboo, treatment with preservatives will improve its resistance to bio-deterioration.

In early developments, preservative standards include inorganic preservatives such as chromate copper arsenate (CCA) and ammoniacal zinc copper arsenate (ACZA), which are detrimental to the environment and human health due to arsenic content [1]. Organic biocides were then employed as alternatives, which have lower mammalian toxicity [2]. Organic biocides such as chlorothalonil have been reported to perform well against brown rot fungus and termites in treating hardwood and softwood [3] but contain toxic cyanide groups, which are not environmental or health friendly.

A new organic formulation, tebuconazole ((RS)-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1H,1,2,4-triazol-1-ylmethyl) pentan-3-ol), also known as TBZ, is a hydrophobic biocide which provides fungal and moulds growth inhibition, pest protection while having lower mammalian toxicity compared to chlorothalonil [4]. Conventional wood preservative treatment involves vacuum pressure treatment of the wood sample while the sample is immersed in a preservative solution. TBZ has water poor solubility which affects its delivery efficiency in vacuum pressure treatment. To enhance delivery efficiency, one novel approach is to encapsulate biocide into nanocarriers *via* the nanoprecipitation method with polymer as part of the formulation. Previous studies also demonstrated that synthetic polymers, such as polylactic acid (PLA), can encapsulate and deliver biocides through vacuum pressure treatment [5]. However, as synthetic polymers are costly and scarce, the current study proposes incorporating tebuconazole into biopolymeric nanocomposites such as chitosan polymeric nanoparticles is proposed.

Chitosan is a renewable polysaccharide sourced from chitin, found in the exoskeletons of crustaceans [6]. Chitosan offers advantages such as biodegradability and nontoxicity as it is sourced from marine origins and has found its use in agriculture, food and biomedical industries [7]. Its application as nano-

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particles (NPs) has been expanding as it is biocompatible and can manage by varying dosage forms, presenting itself as a versatile carrier [8]. Chitosan can also alter its solubility and stability by physical or chemical modification to improve drug delivery. Previous studies on chitosan NPs have been conducted and have shown consistent drug delivery *in vivo*, proving its reliability in delivering human drugs [8]. Chitosan has been reported to exert antifungal activities which can synergise with biocides to enhance preservation [9]. Ding et al. [10] investigated the controlled biocide release using chitosan NPs which reduced leaching from wood, which has demonstrated potential from chitosan NPs in formulating new wood preservation.

In this study, TBZ was incorporated into chitosan NPs *via* the nanoprecipitation method. Synthesized TBZ-loaded chitosan NPs were then used to treat bamboo samples by vacuum pressure. The present study aims to investigate the chemical anti-fungal properties of bamboo strips, to improve the biocide delivery efficiency in water and find out the feasibility of employing chitosan NPs to reduce the cost and biocide dosage required for standard wood treatment.

## EXPERIMENTAL

### Chemicals and Materials

Low molecular weight chitosan polymer (mol wt: approximately 50,000 – 190.000 Daltons based on viscosity) (Aldrich, Iceland), Tebuconazole (93%, Sigma Aldrich, USA), Tween 80 (Fischer Scientific, UK), acetic acid (AR grade, 96-99%) were purchased from respective suppliers. Buluh semantan (*Gigantochloa scortechinii*) specimen and white rot decay fungi (*Trametes versicolor*) were provided by Forest Research Institute Malaysia (FRIM). All materials were used without further purification. Chemical structures of tebuconazole and chitosan are shown in Figure 1.

### Preparation of TBZ-loaded Chitosan NPs

Tebuconazole-loaded chitosan NPs was synthesised using the nanoprecipitation method. Chitosan powder (40 mg) was dissolved in 80% v/v acetic acid (100 ml) while heated at 40 °C and stirred for 3 hours until completely dissolved to form the non-aqueous phase. 10 mg of tebuconazole was dissolved in 10 ml of non-aqueous aliquot with moderate stirring for 5 minutes. The solution was added dropwise (10 mL/hour) to the aqueous phase which consists of 30 mg of Tween 80 surfactant dissolved in 50 mL Milli-Q® ultrapure water with moderate stirring at room temperature. The resulting solution was air dried to remove acetic acid and centrifuged at 12000 rpm for 20 minutes to extract the suspension, followed by washing it with distilled water twice.

### Optimization of TBZ-loaded Chitosan NPs

Optimisation of the NPs is essential to obtain the most efficient formulation for loading TBZ that saves costs and energy. To optimise the NP formulation several variables were investigated: i) the effect of the ratio of chitosan polymer to TBZ, ii) the effect of concentration of chitosan polymer, iii) and the effect of the ratio of solvent phase to aqueous phase were assessed. The adjustments of the variables were by varying i) the mass of TBZ used, ii) the mass of chitosan used, and iii) the amount of Milli-Q® water used respectively.

### Characterization of TBZ-loaded Chitosan NPs

Fourier transform-infrared (FTIR) spectroscopy was employed to characterise chemical structures of bulk TBZ, empty chitosan NPs and TBZ-loaded chitosan NPs from 600 to 4000 $cm^{-1}$ range, analysed with PerkinElmer Frontier FT-NIR spectrometer (USA) under attenuated total reflection (ATR) mode, settings at 4 $cm^{-1}$ with a total of 32 scans. Size distribution and polydispersity were analysed with dynamic light scattering (DLS) via Malvern Zetasizer Nano ZS (UK), with samples dissolved in Milli-Q® water at room temperature, measurement angle set at 173°. Surface morphology of the blank and loaded NPs was analysed with scanning electron micro-scopy (SEM), followed by electron dispersive x-ray (EDX) to analyse the elemental composition of the NPs, using FEI Quanta FEG Type 650 scanning electron microscope with EDX compartment (UK). Samples were freeze-dried, sonicated and coated with platinum paint by Quorum Q150 TS Sputter Coater.

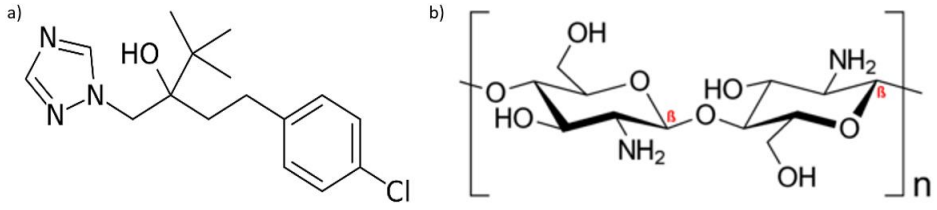


**Figure 1.** Chemical structure of a) tebuconazole (TBZ) and b) chitosan.

UV-visible spectroscopy was used to determine quantitatively TBZ loading efficiency in chitosan NPs using Shimadzu UV-Vis spectrometer (Japan), with range of 200 nm to 600 nm, measuring triplicates and obtaining an average value. TBZ loading efficiency can be determined using equation 1:

$$TBZ\ loading\ efficiency = \frac{Mass\ of\ TBZ\ added - mass\ of\ TBZ\ incorporated}{Mass\ of\ TBZ\ added} \times 100\% \quad (1)$$

**Wood Treatment with TBZ-loaded Chitosan NPs**

Buluh semantan (*Gigantochloa scortechinii*) samples were treated with tebuconazole-loaded chitosan NPs under a full-cell vacuum pressurized system based on Malaysia Standard (MS360:1991). 0.0025% w/v aqueous suspension of NPs was injected into the treatment cell, and the procedure was repeated with 0.0100% w/v aqueous suspension to compare treatability and chemical retention, which can be calculated as using equation 2 and 3:

$$Treatability = \frac{w_{final} - w_{initial}}{G \times V} \times 1000 \quad (2)$$

$$Chemical\ retention = Treatability \times \frac{concentration\ of\ preservative\ solution\ (\%w/v)}{100} \quad (3)$$

Where $w_{initial}$ and $w_{final}$ are the initial and final weights of the bamboo sample, *G* and *V* represent specific gravity of the wood preservative and volume of the bamboo sample. The bamboo samples were dried and analysed for anti-fungi biological efficacy performance studies against white rot decay fungi (*Trametes versicolor*).

RESULTS AND DISCUSSION

**Optimization of TBZ-loaded Chitosan NPs**

Optimisation of the chitosan NPs is required to ensure the most efficient formulation is used for loading TBZ. Hence by looking at the effects of the ratio of chitosan to TBZ, the effect of chitosan concentration, and the effect of the ratio of the solvent phase to the aqueous phase, we can formulate the most efficient and stable composition for the NP for application. Table 2 shows the effect of the ratio of chitosan polymer to TBZ, the effect of concentration of chitosan polymer, and the effect of the ratio of solvent phase to aqueous phase were the parameters for optimisation. In the chitosan polymer to TBZ parameter, results showed that chitosan polymer directly affects the loading of tebuconazole, but the loading effect started to diminish after 1 : 0.5 CHIT:TBZ ratio. This can be attributed to the ionic interactions between hydrogen ions from TBZ and amino groups from chitosan [11], but it will reach the loading saturation of tebuconazole after certain amounts.

**Table 1.** Treatability classification of wood

| Treatability classification | Preservative absorption in 1 $m^{-3}$ timber / $Lm^{-3}$ |
|---|---|
| Very easy | >320 |
| Easy | 240 – 320 |
| Average | 160 – 240 |
| Moderate | 80 – 160 |
| Difficult | <80 |

**Table 2.** Effect of ratio of chitosan polymer to tebuconazole

| Sample | Mass of TBZ (mg) | CHIT:TBZ w/w ratio | $Z_{average}$ (nm) | PDI | TBZ loading efficiency (%) |
|---|---|---|---|---|---|
| **T1** | 5 | 1: 0.125 | 118.55 ± 2.76 | 0.42 ± 0.08 | 38.35 ± 0.01 |
| **T2** | 10 | 1: 0.250 | 194.40 ± 2.01 | 0.29 ± 0.00 | 72.64 ± 0.01 |
| **T3** | 20 | 1: 0.500 | 203.45 ± 2.90 | 0.62 ± 0.15 | 70.68 ± 0.02 |

**Table 3.** Effect of concentration of chitosan polymer

| Sample | Mass of CHIT (mg) | Concentration of CHIT in $H_2O$ (% w/v) | $Z_{average}$ (nm) | PDI | TBZ loading efficiency (%) |
|---|---|---|---|---|---|
| **T4** | 30 | 0.30 | 205.65 ± 7.57 | 0.51 ± 0.32 | 26.33 ± 0.02 |
| **T2** | 40 | 0.40 | 194.40 ± 2.01 | 0.29 ± 0.00 | 72.64 ± 0.01 |
| **T5** | 50 | 0.50 | 182.73 ± 7.74 | 0.52 ± 0.02 | 77.86 ± 0.01 |

Table 3 shows the effect of the concentration of chitosan polymer, results show that as chitosan concentration increased, the nanoparticle hydrodynamic size decreased. One explanation can be due to the higher chitosan concentration increases the viscosity of the solvent phase, reducing the diffusion rate. Higher chitosan concentrations can also form stronger hydrogen bonds between each other due to the presence of amino groups [12]. Increasing chitosan concentration also increases TBZ loading, which may be attributed to the increased surface area of the chitosan NPs and higher chitosan to TBZ ratio.

From the effect of the ratio of the solvent phase to the aqueous phase parameter, it is observed that $Z_{average}$ decreased as the volume of the aqueous phase increased according to results in Table 4. This phenomenon can be explained by the increase in the volume of the aqueous phase which reduces the possibility of agglomeration when achieving supersaturation [13]. One unexpected observation was increasing the aqueous phase ratio decreased TBZ loading efficiency as well. No conclusive understanding may be presented as of now, but it may be attributed to the electrostatic repulsion forces and hydrogen bonding of the NPs.

Based on the 3 tables, T2 has the best PDI value, $Z_{average}$ , TBZ loading efficiency among all the trials. Hence, the formula of T2 is elected as the optimised formula for the chitosan NP synthesis.

**Fourier Transform-Infrared (FT-IR) Spectroscopy**

Blank chitosan NP, TBZ and TBZ-loaded chitosan NP were analysed with FTIR as depicted in Figure 3. In the blank chitosan NP spectrum, bands at 3362 and 3289 $cm^{-1}$ were present and indicate the presence of $N-H_2$ and O-H group stretching vibration [14]. Symmetric and asymmetric C-H stretching can be observed at the band 2874 $cm^{-1}$, and at bands 1644 and 1375 $cm^{-1}$ indicating the presence of C=O stretching and C-N bending vibrations, respectively [14]. Hence, it demonstrated the presence of the amide group in chitosan. Bands at 1065 and 1025 $cm^{-1}$ correspond to the C-O-C bridge chain and stretching vibration of C-O in the cyclic ether group in chitosan [14].

The TBZ spectrum shows O-H stretching band (3288 $cm^{-1}$) and the asymmetric stretching of the aliphatic $-CH_2$ group (2972 $cm^{-1}$), vibration band of N-N (1507 $cm^{-1}$), and C=N (1486 $cm^{-1}$). The presence of C-O (1081 $cm^{-1}$) and C-Cl (676 $cm^{-1}$) stretching vibration was observed, which is consistent with the sterol and chlorophenyl structure [15]. Compared to the blank chitosan NPs, two new bands were observed in the TBZ-loaded chitosan NP spectrum, showing C=N (1533 $cm^{-1}$) and C-Cl (652 $cm^{-1}$) stretching from TBZ. Peaks in the TBZ spectrum were seen in the TBZ-loaded NP spectrum at similar wavenumbers, indicating the successful incorporation of TBZ in the chitosan NP without a reaction between TBZ and chitosan.

By comparing the spectrums of chitosan and TBZ-loaded chitosan NPs, the characteristic C-O stretching peak at 1025 and 1061 $cm^{-1}$ increased in intensity. The C=N (1533 $cm^{-1}$) and C-Cl (652 $cm^{-1}$) stretching has been shifted compared to the bulk TBZ spectrum, where the vibration band of and C=N (1486 $cm^{-1}$) C-Cl (676 $cm^{-1}$) stretching vibration was observed. The slight shift of the bands suggest interaction of TBZ with chitosan, likely due to the hydrogen bonding between the $-NH_2$ and -OH of chitosan and -OH of TBZ, indicating the absence of chemical alteration between chitosan and TEB [14]. Figure 2 proposed a mechanism of the TBZ-loaded chitosan NP formation, where TBZ is adsorbed onto the chitosan NP by intermolecular hydrogen bonding.

**Ultraviolet-visible (UV-Vis) Spectroscopy**

The optimised tebuconazole-loaded chitosan and blank tebuconazole nanoparticles were characterised using UV-Vis Spectrometer. Figure 4 shows the absorption spectra of various concentrations of tebuconazole dissolved in acetic acid. two absorption bands were found, which are at 268 nm and 276 nm, respectively. The occurrence of these absorption bands is attributed to the $\pi \rightarrow \pi^*$ electron transition. Therefore, the $\lambda$ max of the tebuconazole solution was determined at 276 nm [16]. The calibration

curve of TBZ absorbance at 276 nm has an $R^2$ value of 0.9993 which shows a good relationship between absorbance and concentration of TBZ in Figure 5. Intra-day and inter-day assays were carried out for three days to validate the accuracy and precision of the method. Table 5 results show the intra- and inter-day assays were consistent and have insignificant standard deviation, showing good repeatability of the experimental procedure.

**Table 4.** Effect of ratio of solvent phase to the aqueous phase

| Sample | Volume of $H_2O$ (mL) | CHIT : $H_2O$ v/v ratio | $Z_{average}$ (nm) | PDI | TBZ loading efficiency (%) |
|---|---|---|---|---|---|
| **T6** | 25 | 1 : 2.5 | 219.03 ± 7.75 | 0.46 ± 0.06 | 63.52 ± 0.01 |
| **T2** | 50 | 1 : 5..0 | 194.40 ± 2.01 | 0.29 ± 0.00 | 72.64 ± 0.01 |
| **T7** | 100 | 1 : 10.0 | 181.57 ± 4.98 | 0.50 ± 0.05 | 38.35 ± 0.02 |

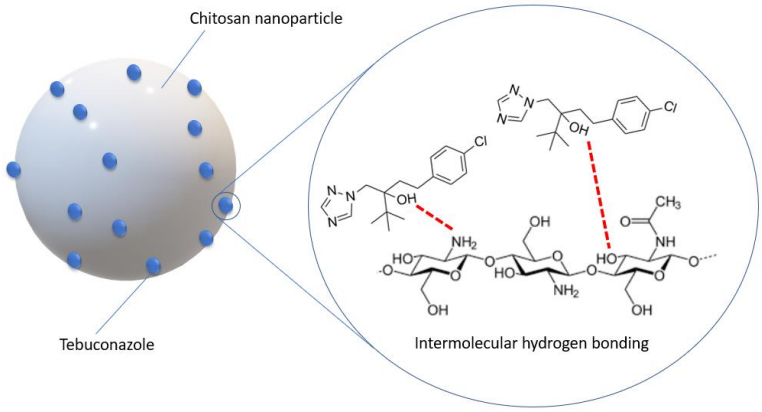


**Figure 2.** TBZ-loaded chitosan NP structure diagram and proposed adsorption mechanism

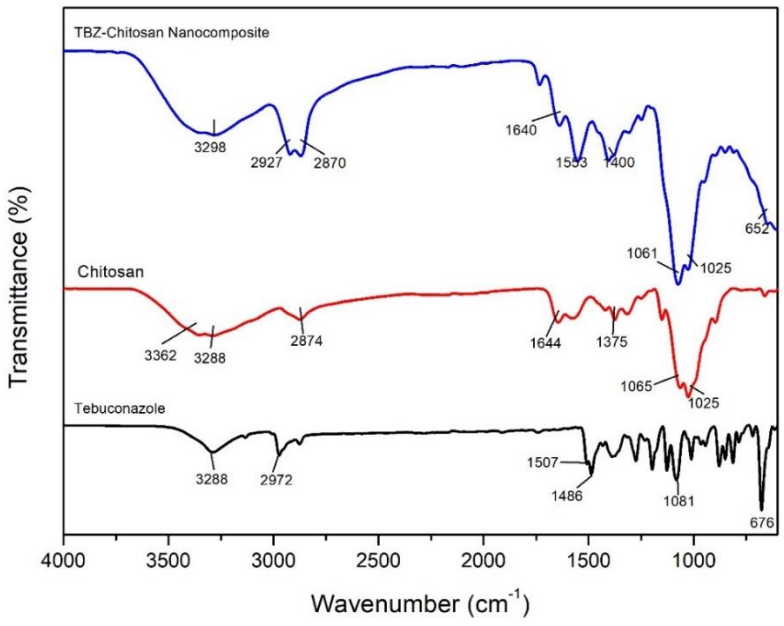


**Figure 3.** FT-IR spectra of TBZ-loaded chitosan nanoparticle, chitosan and tebuconazole

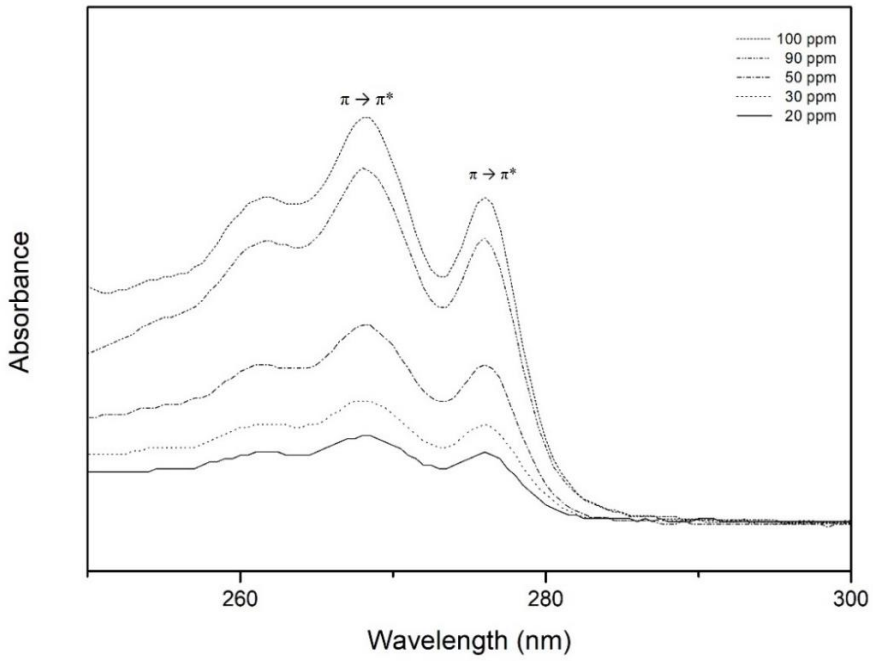


**Figure 4.** Absorption spectra of a series of standard concentrations of TBZ in acetic acid

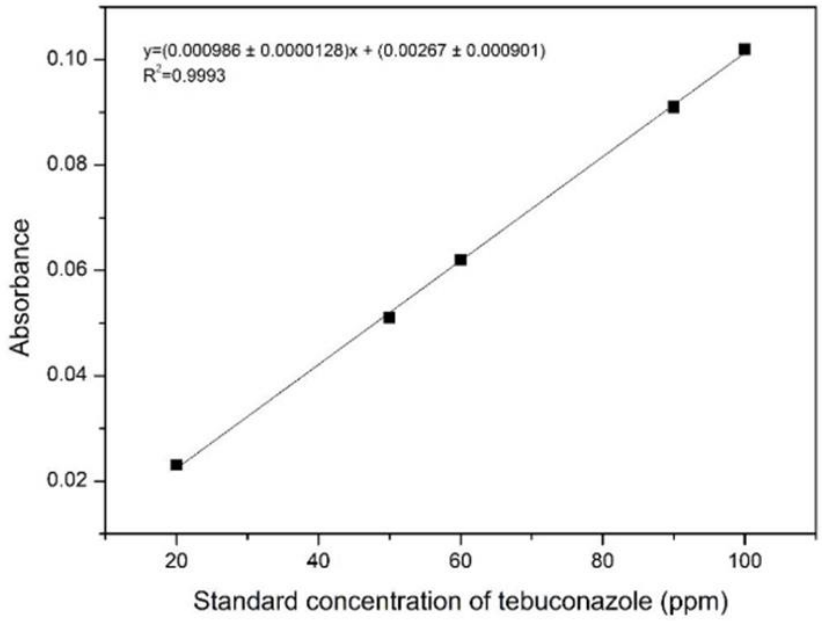


**Figure 5.** Calibration curve of standard concentration of TBZ at a ma ximum wavelength (276 nm)

**Table 5.** Intra-day and inter-day assays: Test of accuracy and precision of the standard concentration of TBZ at maximum wavelength, 276 nm (n = 3)

| Concentration (ppm) | Intra-day | | Inter-day | |
|---|---|---|---|---|
| | Absorbance | %RSD | Absorbance | %RSD |
| 20 | 0.0343 ± 0.00040 | 1.18 | 0.0313 ± 0.0023 | 7.27 |
| 30 | 0.0509 ± 0.00081 | 1.59 | 0.0486 ± 0.0024 | 5.01 |
| 50 | 0.0683 ± 0.00044 | 0.64 | 0.0656 ± 0.0026 | 3.95 |
| 90 | 0.1219 ± 0.00021 | 0.17 | 0.1153 ± 0.0059 | 5.09 |
| 100 | 0.1389 ± 0.00068 | 0.49 | 0.1381 ± 0.0065 | 5.00 |

**Scanning Electron Microscopy-energy-dispersive X-ray Spectroscopy (SEM-EDX)**

The surface morphology of blank chitosan NPs and TBZ-loaded chitosan NPs was investigated with SEM as shown in Figure 6. In general, blank and TBZ-loaded NPs were spherical or oval-shaped, with some irregularly shaped particles that were present. This could be due to the freeze-drying process which possibly deforms the surface from evaporation [17]. The size distribution of blank chitosan nanoparticles demonstrated an average diameter of 91.34 ± 26.4 nm, whereas the obtained average diameter of the tebuconazole-loaded chitosan NPs was 102.23 ± 17.4 nm. From the EDX analysis in Figure 7, the presence of chlorine attributed to TBZ indicates the successful incorporation of TBZ into the chitosan NP.

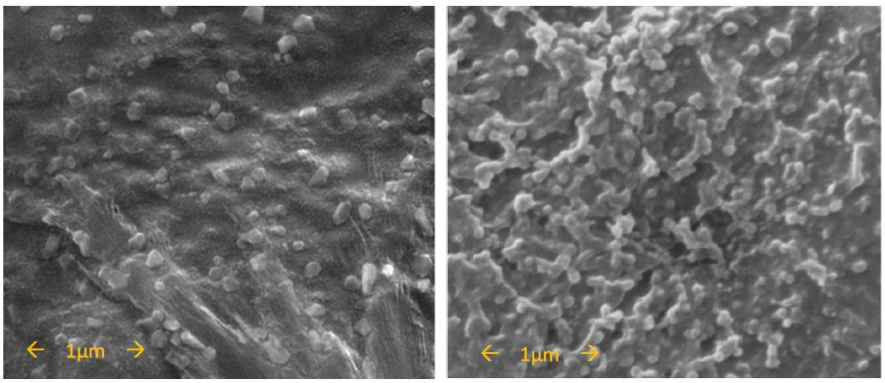


**Figure 6.** SEM micrographs of blank chitosan (left) and TBZ-loaded chitosan NPs (right) at 10000x magnification

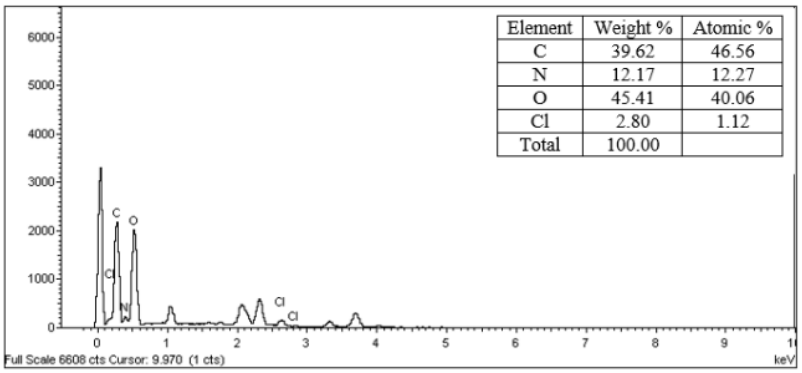


**Figure 7.** Element composition of tebuconazole-loaded chitosan nanoparticles by EDX analysis.

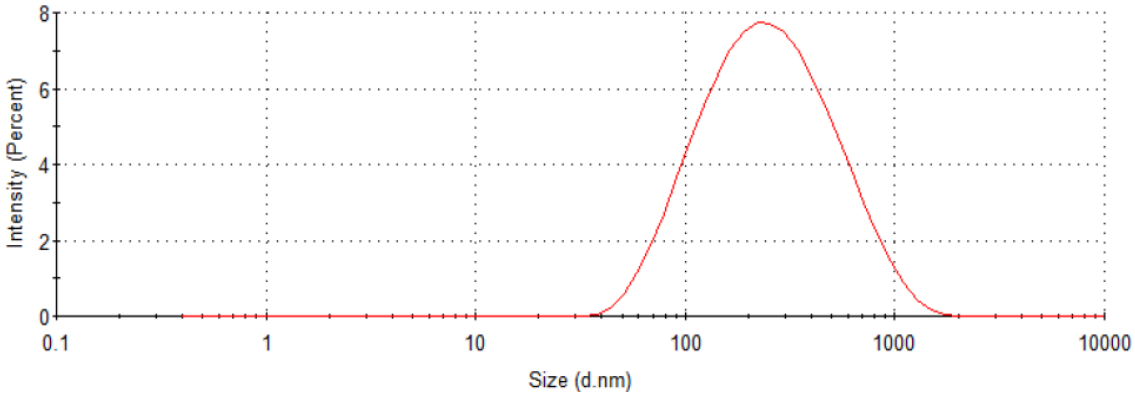


**Figure 8.** Hydrodynamic size distribution of TBZ-loaded chitosan NP

**Dynamic Light Scattering (DLS) Analysis**

The hydrodynamic size distribution of optimized TBZ-loaded chitosan NPs was dissolved in deionized water and measured by DLS analysis in Figure 8. The average hydrodynamic size ($Z_{average}$) of the NPs was 194.4 ± 2.0 nm. The slight enlargement in size of the NPs as compared to SEM (102.23 ± 17.4 nm) was due to the presence of surfactant used in the aqueous phase during the synthesis. The polydispersity index (PDI) was 0.292 ± 0.01, which suggests that the NPs were moderately dispersed. Lower PDI indicates a more homogeneous distribution, which is also an indicator of a stable particle population. Consistent particle size also indicates that no agglomeration occurred during the formation of the NPs as well as the TBZ loading procedure, which is beneficial for the formulation.

**Bamboo Treatment and Efficacy Performance of TBZ-loaded Chitosan Nanoparticles**

Bamboo samples of *Gigantochloa scortechinii* were treated with TBZ-loaded chitosan nanoparticles by full-cell vacuum pressure impregnation treatment, modifying the Malaysian Standard (MS360:1991) which set the pressure at 100 psi for 2 hours. Treatability and chemical retention of the samples were recorded in Tables 6 and 7.

**Table 6.** Treatability of bamboo samples at different concentration of TBZ-loaded chitosan NPs

| Sample | Treatment method | Concentration (%w/v) | Treatability ($Lm^{-3}$) | Treatability class |
|---|---|---|---|---|
| Bamboo treated with TBZ-loaded chitosan NPs | Pressure | 0.0025 | 250.2 ± 8.87 | Easy |
| Bamboo treated with TBZ-loaded chitosan NPs | Pressure | 0.0100 | 226.8 ± 12.19 | Average |

**Table 7.** Chemical retention of wood samples treated with different preservatives

| Sample | Treatment method | Concentration (%w/v) | Chemical retention ($kgm^{-3}$) |
|---|---|---|---|
| Bamboo treated with TBZ-loaded chitosan NPs (This work) | Pressure | 0.0025 | 5.55 ± 0.51 |
| Bamboo treated with TBZ loaded chitosan NPs (This work) | Pressure | 0.0100 | 7.09 ± 0.38 |
| Bamboo treated with chromated copper borate (CCB) [18] | Pressure | 3.50 | 7.2 |
| Bamboo treated with disodium octaborate tetrahydrate [19] | Immersion | 8.00 | 2.2 |
| Bamboo treated with chromated copper arsenate (CCA) [18] | Pressure | 5.00 | 1.49 ± 0.55<br>0.88 ± 0.55 |
| Bamboo treated with TBZ solution [20] | Immersion | 2.0 | *0.25 $kgm^{-2}$ |

Treatability refers to the maximum amount of preservative solution to be absorbed by the bamboo specimen. In both 0.0025% w/v and 0.01% w/v concentrations, the treatability was 250.2 ± 8.87 $Lm^{-3}$ and 226 ± 12.19 $Lm^{-3}$ respectively. The treatability classes were regarded as "Easy" and "Average" respectively. Both concentrations show good treatability and their ability to decent penetration into bamboo, and the procedure can be conducted with a lower concentration of the biocide which optimises energy and materials used for preservation.

In this case, chemical retention refers to the amount of preservative retained in the bamboo specimen. Bamboo samples that were treated with TBZ-loaded chitosan nanoparticles at 0.0025% and 0.01% w/v have chemical retentions of 5.55 ± 0.55 and 7.09 ± 0.38 kg $m^{-3}$, respectively. Relative to other water-based preservatives listed in Table 7, TBZ-loaded chitosan nanoparticles have a better performance at chemical retention while having a less intense treatment method and at reduced fungicide concentration, reducing the energy and cost required for treatment. Comparing to a study by Lee et al. [18] 3.50 % w/v of fungicide was used to achieve a chemical retention of 7.2 kg $m^{-3}$, whereas the TBZ-loaded nano-particles achieved 7.09 ± 0.38 kg $m^{-3}$ with just 0.01 % w/v concentration, showing evident improvement. In a similar study from Sun et al. (2012) bamboo samples were immersed in TBZ solution, and retention was reported to be 0.25 $kgm^{-2}$ in terms of surface area. One explanation of the better performance can be directed at the improved solubility of TBZ as it is incorporated with chitosan. This improves the solubility of TBZ in the water medium where it can penetrate better into the bamboo sample. Pressure treatment for TBZ-loaded chitosan NP also performs significantly better than TBZ solution immersion treatment even at a reduced dosage of biocide. This demonstrates the advantage of pressure treatment as it will cost less material for preservation.

For biological efficacy tests, the treated bamboo samples were tested for resistance against white rot fungi *via* weight loss for 1 month. Based on Table 8, untreated bamboo recorded 22.34 ± 7.97 % weight loss, whereas bamboo samples that were treated at 0.0025% and 0.01% w/v TBZ-loaded chitosan nanoparticles had drastic improvements, recording weight loss at 0.092 ± 0.18 % and 0.026 ± 0.029 % respectively. Both samples are deemed "Highly Resistant".

**Table 8.** Percentage of weight loss of bamboo treated with tebuconazole-loaded chitosan nanoparticles against white-rot fungi (*Trametes versicolor*)

| Sample | Percentage weight loss (%) | Decay rating |
|---|---|---|
| Untreated bamboo | 22.34 ± 7.97 | Resistant |
| Bamboo treated with 0.0025% TBZ-loaded chitosan NPs | 0.092 ± 0.18 | Highly Resistant |
| Bamboo treated with 0.0100% TBZ-loaded chitosan NPs | 0.026 ± 0.029 | Highly Resistant |

## CONCLUSION

TBZ-loaded chitosan NPs have displayed significant antifungal properties in Semantan bamboo samples via pressure treatment, with a marked increase in chemical retention even with reduced biocide concentration. The optimum formulation was found to be 10 mg of tebuconazole in 0.40 %w/v chitosan solution as the solvent phase and 50 mL of ultrapure water as the aqueous phase. Concentration as low as 0.0025% w/v can provide 5.55 ± 0.51 $kgm^{-3}$ of chemical retention of preservative, and result in 0.092 ± 0.18 % weight loss compared to untreated bamboo (22.34 ± 7.97 %) when treated with white-rot fungi (*Trametes versicolor*), displaying strong antifungal activity. TBZ-loaded chitosan NPs provide a good alternative to current preservative systems as it reduces the materials and costs needed to treat bamboo, as well as the environmental impact while providing decent protection against fungi. Further studies on an application for other types of wood are highly recommended to improve current preservative methods.

## ACKNOWLEDGEMENTS

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