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Survival and Feeding Responses of the Formosan Subterranean Termite to Wood Treated with Menadione and Menadione Sodium Bisulfite

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Abstract

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is an invasive species and among the most economically damaging structural pests worldwide. Various insecticides and natural substances have been investigated for their management. Among these, naphthoquinones have demonstrated insecticidal activity against termites and diverse other insect pests. Menadione (vitamin K_3), a naphthoquinone derivative, has been examined for its toxicity and repellency against *C. formosanus* when applied to soil, the tunneling substrate for termites. However, its potential for wood preservation was not investigated. In this study, we tested the water-insoluble menadione and its water-soluble derivative, menadione sodium bisulfite (MSB), for their effects on survival and wood consumption in *C. formosanus* by treating wood for 28 days. Our results revealed the toxic effects of both compounds, with significant termite mortality observed between 500 and 1000 ppm in a no-choice assay. In a choice assay where termites were provided with treated and untreated wood, menadione at concentrations ≥ 500 ppm exhibited a strong feeding deterrent effect and reduced wood damage. However, such effects were not consistently detected for MSB ranging from 1 to 1000 ppm. These findings suggest that menadione is more effective than MSB and may have greater potential as a wood preservative.

Introduction

Subterranean termites (Blattodea: Heterotermitidae) account for approximately 80% of the global economic impact of all termite pests, causing an estimated USD 32 billion per year in treatment and structural repair costs (Rust & Su, 2012). Among them, the invasive Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is one of the most economically damaging termites worldwide (Rust & Su, 2012). Native to eastern Asia (Blumenfeld et al., 2021), this termite is a serious structural pest in Hawaii and the southeastern United States, with annual losses estimated at USD 1 billion in 2005 (Pimentel et al., 2005). This cost has likely increased substantially in recent years due to rising management and structural repair expenses, as well as the continued spread of the termite (Brown et al., 2007; Guidry et al., 2024; Scheffrahn, 2023; Tseng et al., 2021).

A variety of termiticides have been investigated and applied in termite management, including soil treatment, baiting, and wood preservation (Lee & Neoh, 2023; Ohmura & Yamamoto, 2023; Su, 2023). Liquid termiticides are among the most widely used conventional termite control methods and are typically applied to the soil around and beneath the protected structure. These termiticides are categorized into repellents and non-repellents. Compared with repellents, the use of non-repellent termiticides, including fipronil, imidacloprid, chlorfenapyr, and chlorantraniliprole, has been shown to be more effective, resulting in higher termite mortality and more effective protection (Hu, 2011; Lee & Neoh, 2023). Repellent termiticides, mainly pyrethroids, pose challenges because termites avoid treated areas and thorough treatment of all entry points is required; therefore, these compounds are better suited for preventive applications (Hu, 2011; Lee & Neoh, 2023; Su et al., 1982). While repellency may limit their use

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in soil barrier treatments, chemicals with repellent or feeding deterrent effects can be used as wood preservatives instead. For example, bifenthrin and permethrin, which are repellent pyrethroids, are effectively used in the wood preservation industry (Cookson et al., 2009; Guan et al., 2011; Hunt et al., 2005; Lobb & Siraa, 2010; Obanda et al., 2007). Additionally, many naturally derived compounds with insecticidal, repellent, or feeding deterrent activities have been explored, offering alternative options for termite control (Grace, 2023; Oladipupo et al., 2022; Verma et al., 2009).

Menadione (2-methyl-1, 4-naphthoquinone), also known as vitamin K_3, is a water-insoluble compound and a precursor to biologically active forms of vitamin K (Abdelmohsen et al., 2004). This compound has a low level of mammalian toxicity (0.5 g/kg orally in mice) (O'Neil et al., 2001). Although not allowed in human food, both menadione and its water-soluble derivative, sodium menadione bisulfite (MSB), have been used as an inexpensive nutritional supplement in animal feeds (Speek et al., 1984). These synthetic naphthoquinone derivatives and naturally occurring naphthoquinones show insecticidal properties against various urban and agricultural pests, suggesting their use as safe and cost-efficient pest control agents (Khambay & Jewess, 2000). For example, plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) has demonstrated strong acute toxicity against the house fly (*Musca domestica* Linnaeus) (Pavela, 2013). 5-Hydroxy-2-methyl-1,4-naphthoquinone isolated from the roots of persimmon (*Diospyros kaki* Thunberg), as well as its structural derivatives, were reported to have potent larvicidal activities against three mosquito species (*Aedes aegypti* L., *Culex pipiens* L., and *Ochlerotatus togoi* Theobald) (Kim & Lee, 2016). Similarly, MBS exhibited insecticidal properties against the sweet potato whitefly *Bemisia tabaci* (Gennadius) and the tomato psyllid *Bactericera cockerelli* (Sulc) (Delgado Ortiz et al., 2022). In addition, both toxic and repellent effects of naphthoquinones extracted from the heartwood of teak (*Tectona grandis* L.f.) have been demonstrated on the drywood termite *Incisitermes marginipennis* (Latreille) (Velázquez-Becerra et al., 2023). Menadione and other naphthoquinones presumably act by disrupting mitochondrial respiration in insects, as evidenced by their uncoupling of mitochondrial oxidative phosphorylation and cytotoxic activities in mammalian cells (Brière et al., 2004; Majiene et al., 2019).

Our previous study evaluated the effects of menadione on *C. formosanus* through soil treatment, and the results showed both strong toxic and repellent activities (Ngo et al., 2021). When menadione at 6-600 ppm was applied to sand, 90-100% mortality in seven days was observed in a no-choice assay; in a choice assay where termites were exposed to treated and untreated sand, menadione at ≥ 6 ppm showed significant repellent activity and prevented termites from moving to the treated side for feeding (Ngo et al., 2021). Although repellency is a less desirable property for soil termiticides than non-repellency, investigating menadione for its potential use as a wood preservative remains valuable. In this study, we examined the effects of menadione and MSB on the survival and feeding of *C. formosanus* termites over a 28-day period via wood treatment. First, a no-choice assay was conducted to evaluate the toxicity and the effects on the consumption of wood treated with each compound at concentrations ranging from 0 to 1000 ppm. Additionally, a choice assay comparing untreated and treated wood was conducted to assess the potential feeding-deterrent effects of menadione and MSB.

Materials and Methods

Insects

Workers and soldiers from *C. formosanus* colonies were collected at Brechtel Park, New Orleans, Louisiana, using pine wood traps buried in the ground (Gautam & Henderson, 2011). The collected termites were then maintained in a laboratory at 25 ± 1 °C and kept in complete darkness in acrylic plastic containers (45.7 × 30.4 × 22.8 cm, Rubbermaid, Atlanta, GA). Organic soil (Miracle-Gro All Purpose for In-Ground Use, Scotts Miracle-Gro, Marysville, OH, USA) was placed at the bottom, and moistened pine wood blocks were provided as the food source. The termites were used for experimentation after a two-week acclimation period in the laboratory.

No-Choice Assay

A no-choice assay was conducted to test whether wood treated with either menadione or MSB at different concentrations affected the mortality and wood consumption of *C. formosanus* termites. Kiln-dried pine wood blocks (*Pinus* sp., purchased from the local Home Depot store) were cut into pieces (3.6 × 1.7 × 0.6 cm), oven-dried at 100 °C for 24 hours, and weighed. The wood pieces were then immersed in solutions of menadione or MSB (≥ 98.0% and ≥ 95.0%, respectively, MilliporeSigma, Burlington, MA, USA) at concentrations of 0 (solvent control), 1, 10, 100, 500, and 1000 ppm for 30 seconds. The concentrations were selected based on a previous study (Ngo et al., 2021), which demonstrated strong dose-dependent effects of menadione on termite survival, sand penetration, and feeding at concentrations extending into the high ppm range. Menadione solutions were prepared with acetone as the solvent, while MSB solutions were prepared with distilled water. For the menadione treatment, the immersed wood pieces were dried in the fume hood for 24 hours to facilitate acetone evaporation before use in experiments.

The assay was conducted in round plastic containers (3.6 cm in height and 5.0 cm in diameter, Pioneer Plastics, North Dixon, KY, USA) (Fig 1A). Each container was filled with 60 g of sand (Quikrete Premium Play Sand, Atlanta, GA, USA), and 9 mL of distilled water was added to reach 15% moisture. To prevent any leaching into the sand, each treated wood piece was placed on an aluminum foil sheet (4.1 cm in length and 2.5 cm in width), which was then positioned on top of the sand. A group of 100 termites (90 workers and 10 soldiers) was released into each container, which was then covered with a lid. The experimental containers were placed inside a larger sealed plastic container (25.4 × 17.8 × 7.6 cm), where moistened paper towels were provided at the bottom to maintain about 98% relative humidity (RH). The experiment was conducted for 28 days at room temperature (25 ± 1 °C) in complete darkness. At the end of the assay, termite mortality was recorded, and wood consumption was calculated based on the weight difference of the wood after oven-drying at 100 °C for 24 hours. Termites from three *C. formosanus* colonies were used, with each colony replicated three times using different groups of termites.

Choice Assay

A choice assay was conducted to determine if menadione or MSB exhibited any deterrent effects on *C. formosanus* termites through wood treatment. The assays were conducted in glass jars (9.7 cm in height and 8.5 cm in diameter; Qorpak, Clinton, PA, USA) (Fig 1B). Each jar contained 350 g of sand, with 52.5 mL of distilled water added to reach 15% moisture content. Menadione and MSB solutions at concentrations of 0 (solvent control), 1, 10, 100, 500, and 1000 ppm were prepared to treat pine wood pieces as described above. In each jar, two wood pieces (one untreated and one treated) were placed on top of the sand, each held separately by an aluminum foil sheet (4.1 cm in length and 2.5 cm in width) to prevent leaching into the sand. A group of 100 termites (90 workers and 10 soldiers) was then introduced to each jar. The jars were covered with a lid, slightly loosened to allow ventilation.

The experimental glass jars were placed in a larger sealed container (31.8 × 25.6 × 9.8 cm) with a moistened paper towel at the bottom to maintain an RH of about 98%. The experiment was conducted for 28 days at room temperature (25 ± 1 °C) in complete darkness. At the end of the assay, termite mortality was recorded, and wood consumption of each piece was calculated by pre- and post-experiment weight difference of the wood, which was oven-dried at 100 °C for 24 hours. Termites from three *C. formosanus* colonies were used, with each colony replicated three times using different groups of termites.

Data Analysis

Data were analyzed using the R software v4.3.2 (the R Foundation, Vienna, Austria) (Ihaka & Gentleman, 1996) and visualized using PRISM v10.1.2 (GraphPad Software, San Diego, CA, USA). Since assays for menadione and MSB were conducted at different times with slightly different treatment methods, the data for the two compounds were analyzed separately. Additionally, in the no-choice assays, data from several replicates were excluded due to fungal growth in the early stages, resulting in varying sample sizes across treatments (menadione: $n = 8$ for 0, 500 and 1000 ppm, $n = 7$ for 1 ppm, and $n = 9$ for 10 and 100 ppm; MSB: $n = 8$ for 0 ppm, $n = 7$ for 1 ppm, and $n = 9$ for all other treatments).

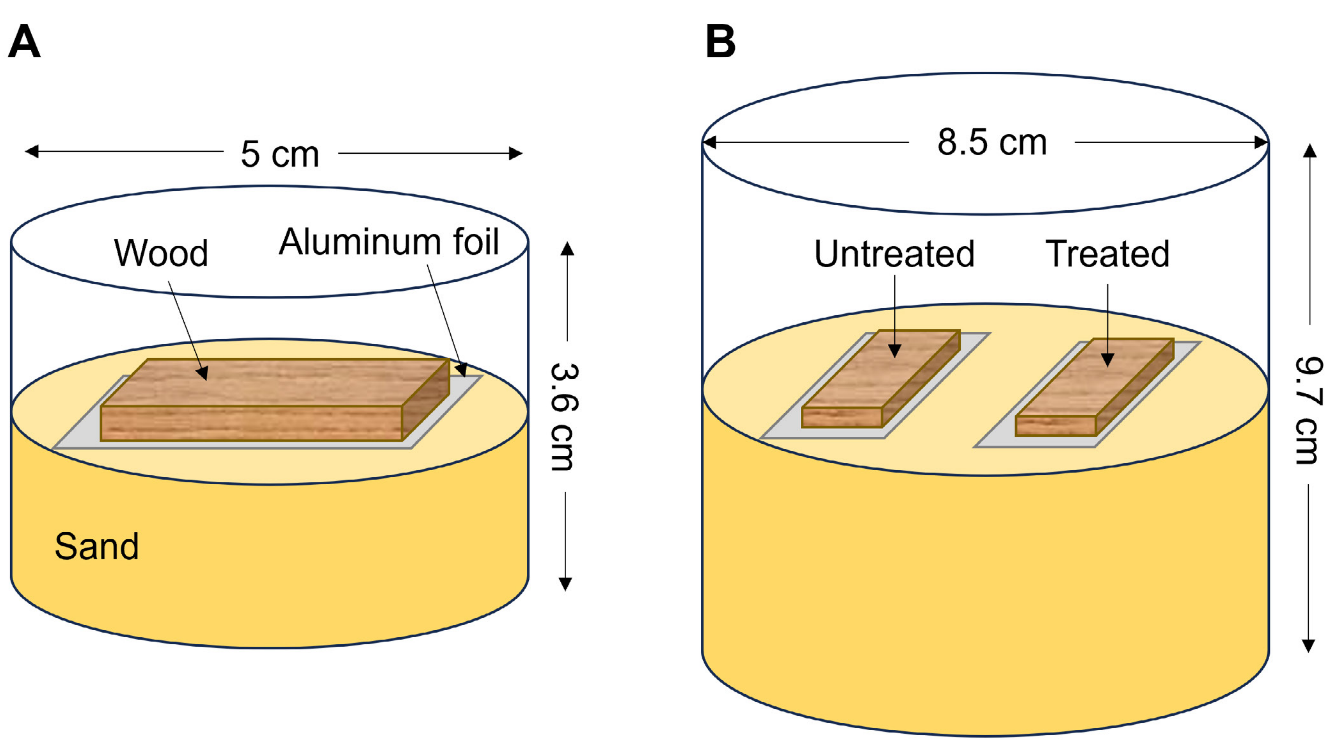

Fig 1. Schematic illustration of experimental setups. (**A**) No-choice assay; (**B**) Choice assay.

For all data, normality was tested using Shapiro-Wilk tests, and homogeneity of variance was evaluated using Levene's test ($\alpha = 0.05$). As the data did not meet the assumptions for parametric analyses, a generalized linear mixed model (GLMM) was used to analyze mortality data from all assays and wood consumption data from the no-choice assays. Colony was coded as a random factor and treatment as a fixed factor, and GLMM was followed by mean separation tests using the *lsmeans* package. The Wilcoxon signed-rank test was performed for pairwise comparisons of wood consumption data from the choice assays. The original data are provided in the supplemental materials (Table S1).

Results

No-Choice Assay

Menadione affected the survival and wood consumption of *C. formosanus* termites through wood treatment. At 500 ppm, significantly higher termite mortality (35.88 ± 11.18%, mean ± SE) compared to the control (0 ppm, 9.62 ± 1.94%) was observed (GLMM, $df = 41$, $p < 0.05$, Fig 2A). Although mortality at 1000 ppm (27.50 ± 10.41%) was higher than that at 0, 1, 10, and 100 ppm, the differences among these groups were not statistically significant (GLMM, $df = 41$, $p > 0.05$, Fig 2A). Additionally, termites consumed significantly less wood treated with 1000 ppm of menadione (0.06 ± 0.02 g) compared to the control (0.23 ± 0.05 g) and concentrations ranging from 1 to 100 ppm (GLMM, $df = 41$, $p < 0.05$, Fig 2B).

Similar to menadione, MSB affected termite mortality in a concentration-dependent manner. At 1000 ppm, significantly higher termite mortality (75.56 ± 10.42%) was observed compared to the 0 ppm control (12.13 ± 3.46%) (GLMM, $df = 43$, $p < 0.05$, Fig 3A). Termite mortality did not significantly differ between the control and MSB treatment at concentrations from 1 to 500 ppm (GLMM, $df = 43$, $p > 0.05$, Fig 3A). Wood consumption at 1000 ppm of MSB (0.09 ± 0.04 g) was lower than that in the control group (0.19 ± 0.04 g); however, no significant differences were observed between these groups or across other tested concentrations (GLMM, $df = 43$, $p > 0.05$, Fig 3B). Representative images of wood damage observed in the no-choice assays for menadione and MSB are presented in Fig 4.

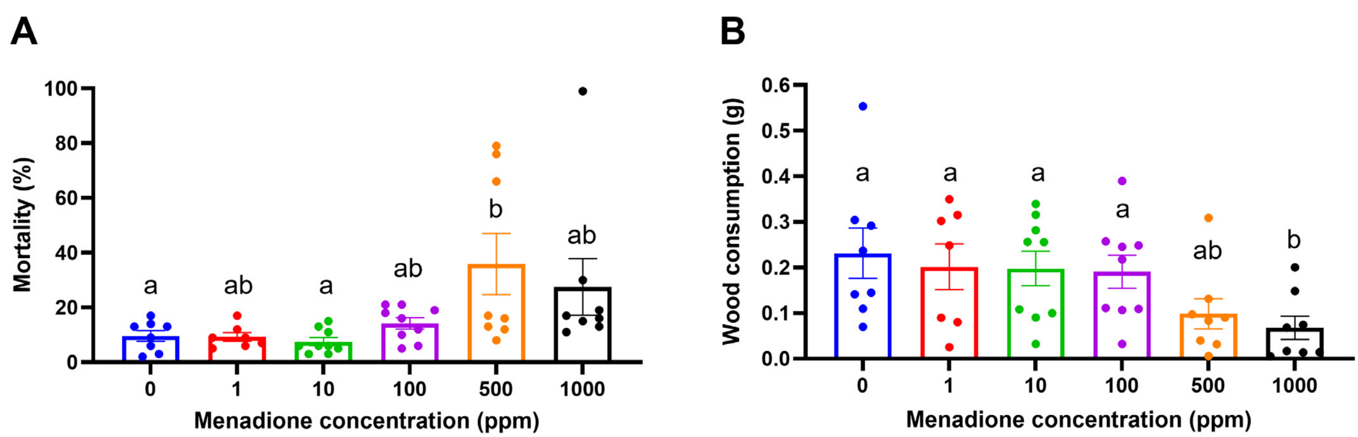

Fig 2. Effects of menadione on termite mortality and feeding in the no-choice assay. (**A**) Termite mortality after 28 days of treatment. (**B**) Wood consumption after 28 days of treatment. Groups sharing the same letter are not significantly different (GLMM, $df = 41$, $p > 0.05$). Bars represent mean ± SE, and dots show individual values.

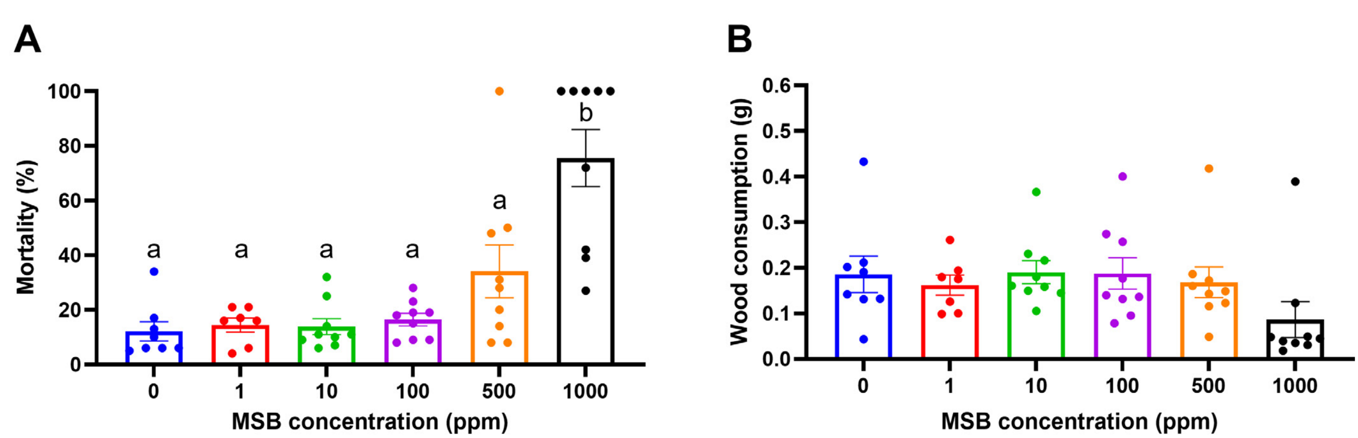

Fig 3. Effects of MSB on termite mortality and feeding in the no-choice assay. (**A**) Termite mortality 28 days post-treatment. Groups sharing the same letter are not significantly different (GLMM, $df = 43$, $p > 0.05$). (**B**) Wood consumption over 28 days post-treatment. No significant differences were detected among groups (GLMM, $df = 43$, $p > 0.05$). Bars represent mean ± SE, and dots show individual values.

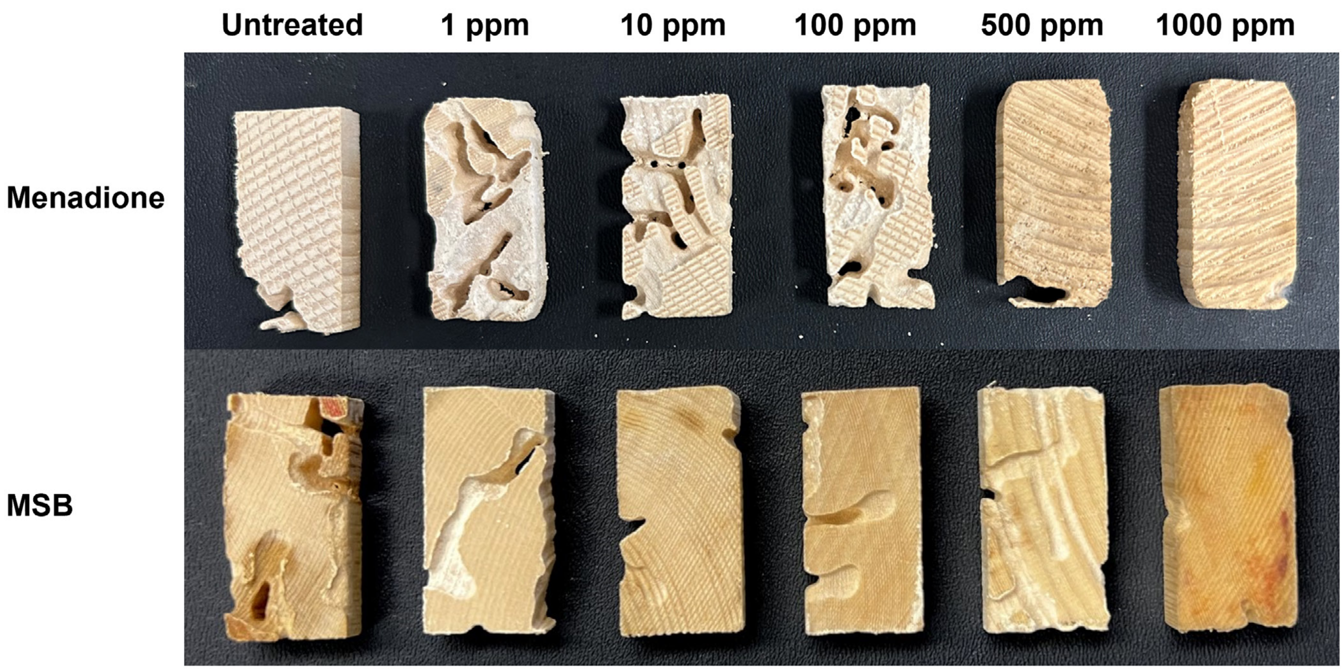

Fig 4. Representative images of wood damage by termites 28 days post-menadione and MSB treatments in no-choice assays.

Choice Assay

When termites were provided with both an untreated and a menadione-treated wood piece, overall mortality did not differ significantly across the tested concentrations (GLMM, $df = 46$, $p > 0.05$, Fig 5A), with mean mortality below 18% in each group. Wood consumption did not differ significantly between untreated and treated wood at menadione concentrations ≤ 100 ppm (Wilcoxon signed-rank test, $p > 0.05$, Fig 5B). However, consumption was significantly reduced for wood treated with menadione at 500 and 1000 ppm (0.04 ± 0.01 and 0.03 ± 0.01 g, respectively) compared to the paired untreated pieces (0.21 ± 0.05 and 0.18 ± 0.04 g, respectively) (Wilcoxon signed-rank test, $p < 0.01$, Fig 5B).

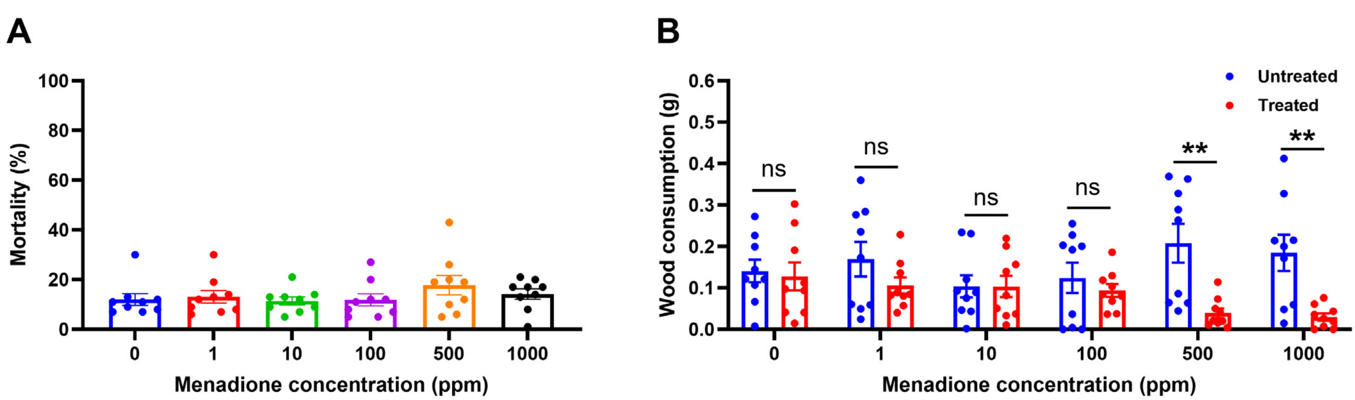

Fig 5. Effects of menadione on termite mortality and feeding in the choice assay. (**A**) Termite mortality 28 days post-treatment. No significant differences were detected among groups (GLMM, $df = 46$, $p > 0.05$). (**B**) Wood consumption over 28 days post-treatment (Wilcoxon signed-rank test; ns: not significant ($p > 0.05$); ** $p < 0.01$). Bars represent mean ± SE, and dots show individual values.

In the choice assay with MSB, overall termite mortality was significantly higher at the concentration of 1000 ppm (48 ± 10.39%) than other groups (GLMM, $df = 46$, $p < 0.05$), while no significant differences were found among the other concentrations from 0 to 500 ppm (GLMM, $df = 46$, $p > 0.05$, Fig 6A), where mean mortality remained below 15%. A significant reduction in the consumption of treated wood compared to untreated wood was observed only at 100 ppm (Wilcoxon signed-rank test, $p < 0.05$), while no significant differences in consumption of paired wood pieces were found at other concentrations (Wilcoxon signed-rank test, $p > 0.05$, Fig 6B). Representative images of wood damage observed in the choice assays for menadione and MSB are presented in Fig 7.

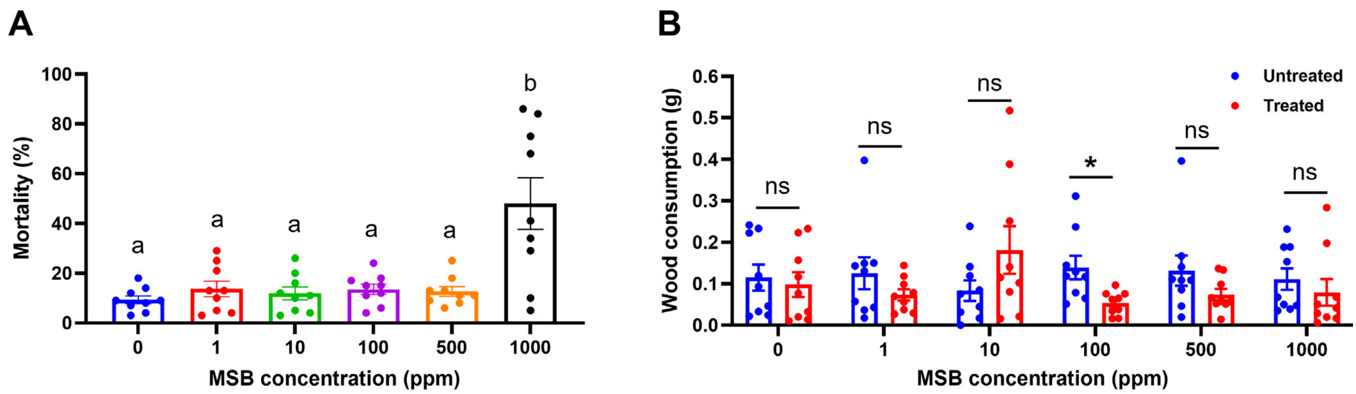

Fig 6. Effects of MSB on termite mortality and feeding in the choice assay. (**A**) Termite mortality 28 days post-treatment. Groups sharing the same letter are not significantly different (GLMM, $df = 46$, $p > 0.05$). (**B**) Wood consumption over 28 days post-treatment (Wilcoxon signed-rank test; ns: not significant ($p > 0.05$); $*p < 0.05$). Bars represent mean ± SE, and dots show individual values.

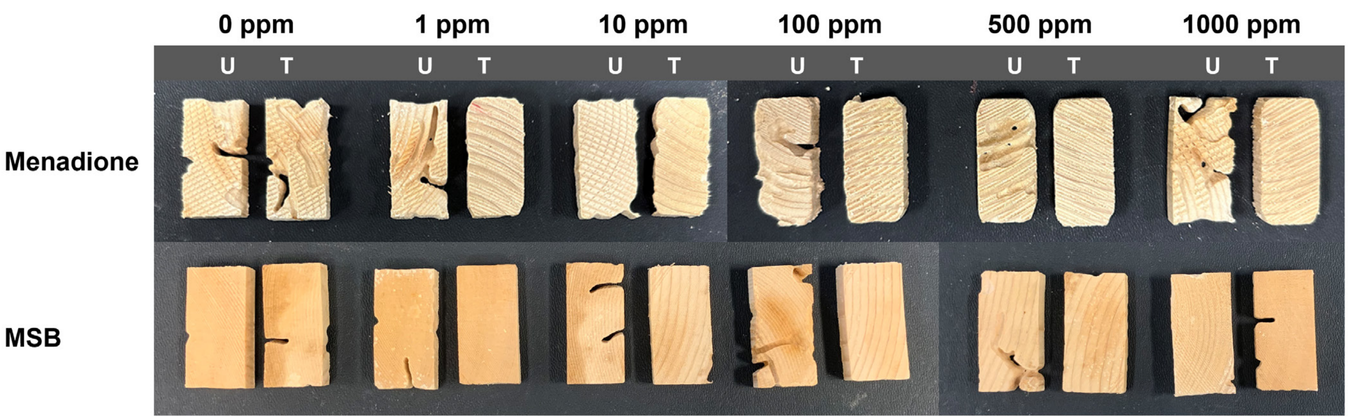

Fig 7. Representative images of wood damage by termites post-menadione and MSB treatments in choice assays. U: untreated; T: treated.

Discussion

In this study, both menadione and MSB caused mortality in *C. formosanus* termites; however, their effects differed. While menadione at 1000 ppm resulted in less than 30% mortality and this effect was not statistically significant, feeding was reduced by more than 70% compared to the untreated control (Fig 2). In contrast, MSB at the same concentration resulted in much higher mortality (about 75%) but only reduced feeding by 52% (Fig 3). Additionally, the choice assay demonstrated a strong feeding deterrent effect of menadione at 500 and 1000 ppm without causing significant mortality (Fig 5), whereas MSB did not consistently show deterrent activity at the tested concentrations (Fig 6). The results suggest that menadione is more effective than MSB in wood protection due to its feeding deterrence. This compound may be considered for protection against termite damage to structural wood, wooden furniture, and other cellulose-containing materials, such as artwork, pending further testing and optimization.

The concentrations of menadione and MSB evaluated in this study (extending to 500-1000 ppm) were selected based on a previous soil treatment study (Ngo et al., 2021), with the aim of further evaluating termite responses to surface-treated wood under laboratory conditions. Direct comparisons with commercial wood preservatives are complicated by differences in formulation and treatment method. For example, borate-based preservatives typically achieve boron retention levels of ≥1% boric acid for termite protection, with higher retentions often required under severe termite infestations or for long-term performance (Freeman et al., 2009). These levels are achieved through pressure treatment or diffusion-based processes that ensure deeper, more uniform penetration into the wood. In contrast, the concentrations of menadione evaluated here (500–1000 ppm) were applied via short-term surface immersion and are therefore not directly comparable to industrial preservative retentions. Therefore, menadione is not proposed as a stand-alone replacement for existing commercial wood preservatives at this stage, but rather as a potential supplementary feeding deterrent that could be incorporated into integrated termite management strategies to help reduce wood damage.

Compared to our previous study, where soil-treated menadione at 6 ppm triggered significant termite mortality in a no-choice assay and avoidance in a choice assay (Ngo et al., 2021), this study required a higher concentration of 500 ppm to observe similar effects with wood treatment. This difference is likely due to varying levels of termite exposure to the treatments between the two studies. When only the wood was treated, termites were exposed to the tested chemicals solely through feeding, without contact during tunneling in the substrate sand. Previous research on non-repellent termiticides, such as fipronil, showed that termites can transfer these chemicals to other colony members, leading to increased mortality and potential colony suppression (Ibrahim et al., 2003; Itakura et al., 2021; Vargo & Parman, 2012). However, such horizontal transfer is less likely to occur with repellent chemicals, and lower mortality is generally expected when termites have access to untreated areas (Hu, 2011), as observed with menadione treatment in this case. Additionally, the diffusion of menadione and MSB is yet to be examined. It is possible that the chemicals did not penetrate the wood after soaking for 30 seconds, and the observed effects might likely reflect surface level exposure rather than fully impregnated wood. Future studies should adopt standardized wood preservation methods, such as pressure treatment, extended soaking duration, or multiple treatment cycles, to improve penetration and enable a more rigorous evaluation of preservative efficacy under conditions relevant to structural wood protection (Ohmura & Yamamoto, 2023). Moreover, although the pine wood used in this study is among the most commonly used structural wood species, treatment optimization requires testing additional wood species. Also, wood exhibits natural non-uniformity even within the same species, and heterogeneities such as variations in grain patterns, density, and proportions of early- and latewood may influence treatment outcomes.

In termite management, toxicity, repellency, and feeding deterrence elicit mechanistically distinct responses with different implications for wood protection. While toxicity leads to mortality following exposure, repellency involves avoidance of treated materials prior to contact, and feeding deterrence reflects reduced consumption despite contact with the treated substrate. Menadione may act solely as a feeding deterrent, a repellent, or both to *C. formosanus*, which was not distinguished in this study and warrants further investigation through tracking termite behavior. At 500 ppm, menadione treatment significantly reduced but did not completely eliminate wood consumption in the choice assay (Fig 5B). Along with the low volatility of menadione, this suggests that direct contact with the chemical was necessary for *C. formosanus* termites to stop feeding, potentially due to perception via their olfactory and gustatory systems (Chapman, 2003; Sun, 2023). From a practical perspective, however, the outcome is the same: termites can be deterred from consuming the treated wood.

Wood treated with MSB at 1000 ppm exhibited a lethal effect on *C. formosanus* termites in both no-choice and choice assays, but this mortality did not result in significant reductions in wood damage (Fig 3 and 6). MSB may be slow-acting, with termite mortality occurring toward the end of the 28-day assays. The results imply that MSB is non-repellent, or that it may require higher doses to exhibit repellency or feeding deterrence, warranting further investigation. MSB has previously been shown to have insecticidal activity against agricultural pests (Delgado Ortiz et al., 2022; Enriquez et al., 2024), but its effect on insect feeding has not been reported. While this study did not provide strong evidence supporting the use of MSB as a wood preservative, MSB may be considered for testing as a soil termiticide. Additional studies will be necessary to determine whether this compound is toxic, slow-acting, and non-repellent when applied to soil.

Furthermore, compared to MSB, which easily dissolves in aqueous solutions, menadione is less likely to leach out of wood because of its water-insoluble nature. Leachability is an issue associated with water-soluble chemicals, which prevents their effective use in treating wood for exterior use or for use in contact with the ground (Obanda et al., 2008). For example, borates are widely used as wood preservatives due to their broad-spectrum effectiveness and environmentally friendly properties; however, their use is limited to above-ground environments protected from weather exposure (Obanda et al., 2008). Additional coating or fixation of these compounds would be necessary to reduce leaching (Furuno et al., 2003; Obanda et al., 2008). Importantly, while water-insolubility is a favorable characteristic of menadione for topical use, further investigations are needed to optimize its formulation and to evaluate its residual effects under varying environmental conditions, such as temperature and light.

Another important future direction is to examine the effects of menadione and MSB on other termites and wood-destroying insects. It is worth noting that insecticide susceptibility can vary substantially among termite species (Lee & Neoh, 2023). For example, compared with *Reticulitermes*, *C. formosanus* is more tolerant to bifenthrin but susceptible to imidacloprid with multi-fold differences in topical LD_{50} (Mao et al., 2011; Rust & Saran, 2008). The topical application of chlorfenapyr, which has a similar mode of action to menadione, showed *C. formosanus* to be more susceptible than *R. hesperus* (Mao et al., 2011; Rust & Saran, 2006). Additionally, because subterranean termites increase moisture in wood while feeding or foraging, it is important to evaluate the antifungal properties of potential wood preservatives to ensure protection against wood-decaying fungi. Since menadione interferes with mitochondrial oxidative phosphorylation (Brière et al., 2004; Majiene et al., 2019), a process highly conserved across eukaryotes (Gnad et al., 2010), it will be valuable to further determine whether this compound and its derivatives exhibit broad-spectrum effects and how their efficacy varies among structural pests.

Supplemental Materials: Table S1: Original data.

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Conflicts of Interest: The authors declare no conflicts of interest.

Authors' Contribution

A.K.: Data curation, formal analysis, investigation, methodology, visualization, writing-original draft, writing-review and editing.
M.G.: Data curation, formal analysis, investigation, funding acquisition, writing-review and editing;
R.L.: Conceptualization, writing-review and editing.
Q.S.: Conceptualization, methodology, project administration, resources, funding acquisition, supervision, validation, writing-review, visualization and editing.

All authors gave final approval for publication.

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