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RESEARCH PAPER

The toxicity of ammonia as a fumigant to dry wood termite (*Cryptotermes cynocephalus* L.) on sengon wood

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ABSTRACT

Wood-based packaging material is usually made from low-quality kind of wood, making it a potential medium for carrying or housing wood-destructive organisms. The objective of this research was to investigate the toxicity of ammonia as a fumigant for controlling the dry-wood termite, *Cryptotermes cynocephalus*. Ammonia was applied as ammonium hydroxide (NH_4OH) 25%, which was placed inside the fumigation chamber. At 2.25 cm wood thickness, toxicity tests were performed using 3 levels of NH_4OH doses (0, 700, and 3800 mL/m³) and 4 levels of exposure (4, 6, 8, and 10 hours). The toxicity of ammonia fumigant at 1.25 cm wood thickness was assessed using 6 dose levels of NH_4OH (0, 200, 378, 587, 1732, and 4188 mL/m³) for 4 hours of exposure. The LD_{50} and LD_{90} values of ammonia against the dry-wood termite, *C. cynocephalus*, inside the sengon wood at 2.25 cm thickness after 4 hours of exposure were 3263 mL NH_4OH/m³ and 22,781 mL NH_4OH/m³, respectively. Moreover, for 1.25 cm wood thickness, for the same exposure duration (4 hours), the LD_{50} and LD_{90} values of ammonia fumigant were 541.594 mL NH_4OH/m³ and 1432.125 mL NH_4OH/m³, respectively. Meanwhile, for 0.25 cm wood thickness, the LD_{50} and LD_{90} values were 67.67 mL of NH_4OH/m³ and 241.140 mL NH_4OH/m³, respectively. In addition, for 2.25 cm wood thickness, the LD_{50} and LD_{90} values of ammonia fumigant were lower when the exposure time was longer (2149 and 10,722 mL NH_4OH/m³ for 6 hours of exposure, and 1373 and 8705 mL NH_4OH/m³ for 8 hours of exposure).

Key words: ammonia, doses, dry wood termites, toxicity

INTRODUCTION

Wood packaging is widely used in domestic and international trade. The package has to meet some requirements, such as dimension, raw materials, quality, and phytosanitary standards (Frąś et al., 2018). The raw material for wood packaging in Indonesia is sourced from plants originating from industrial plantation forests and community forests, which are vulnerable to attack by wood-destroying organisms (WDO) (Rismayadi, 2008).

Wood packaging is generally made from various types of raw and low-quality wood (softwood) (Krishnankutty et al., 2020). According to Surjokusumo (2005), most of the wood used as packaging qualified as class III-V durability, which has low economic value , making it very vulnerable to attacks by WDO.

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This condition causes the wood packaging to have the potential to become a source and carrier of wood-destroying organisms, especially wood-boring insects and several types of fungi and nematodes. Some wood-destroying insects found in wood packaging include *Aromia moschata, Callidium coriaceum, Hylotrupes bajulus, Callidium violaceum, Sirex juvencus, Urocerus gigas gigas, Anoplophora chinensis, Aromia* sp.*, Batocera lineolata, Hesperophanes* sp.*, Monochamus alternatus, Phoracantha semipunctata, Purpuricenus temminckii, Saperda* sp.*, Xylotrechus* sp.*,* and *Cryptotermes* sp. (Eyre et al., 2018).

Cryptotermes sp., known as termites, live in wooden structures, making it difficult to detect and control them. In addition to damaging dry wood, *Cryptotermes* sp. is also reported as a pest found in many plantation crops, such as pepper in the Lampung area (Hariri et al., 2003). *C. cynocephalus* belongs to the Phylum Arthropoda, Class Insecta, Order Isoptera, Family Kalotermitidae, and Genus Cryptotermes (Nandika et al., 2003). These termites live in wooden structures and build colonies that cause damage to wood products.

However, until now, the type and dose of fumigant that are effective for controlling these termites are not known. It is reported that termites in wood packaging

can be controlled by fumigation using sulfuryl fluoride and/or methyl bromide. The results of Alfian's research (2015) showed that fumigation using sulfuryl fluoride can result in *C. cynocephalus* mortality values of up to 100% at a wood thickness of 10 cm, with a dose of 15–30 g/m^3 and an exposure time of 24 hours. Fumigation with methyl bromide for wood packaging uses a dose of 32 g/m^3 with an exposure time of 24 hours at temperatures above 21 ºC to eliminate all insects (Agricultural Quarantine Agency, 2007a).

The 2009 International Standard for Phytosanitary Measure (ISPM #15) for wood packaging (Guidelines for Regulating Wood Packaging Materials in International Trade) is a guideline for implementing plant quarantine measures for wood packaging. Based on these regulations, wood packaging must go through methyl bromide fumigation or heat treatment, as well as labeling. The use of methyl bromide as a fumigant has been evaluated for its potential as an ozone-depleting agent. The Montreal Protocol 1999 (UNEP, 2000) mandated the elimination of the use of methyl bromide in developed countries in 2005, while for developing countries, it was set in 2015 except for quarantine and pre-shipment purposes because there was no technically and economically viable alternative substitute (Hidayat, 2012).

Following up on this, it is necessary to carry out research on alternative fumigants to control wood pests as a substitute for methyl bromide. One of the materials that can be used as a fumigant is ammonia (Wahyudi et al., 2012). Ammonia is a colorless, pungent smelling gas with a boiling point of -33.5 ºC. The liquid has a free heat of vaporization of 1.37 kJ/g at its boiling point and can be handled with laboratory equipment (Appl, 1999). Ammonia is widely used in wood preservation; fumigation with ammonia can reduce wood moisture content, improve wood quality, and increase the mechanical density of wood (Hackenberg et al., 2021). Ammonia fumigation on wood can improve wood quality (Azhim, 2011). The toxicity of ammonia to living things can be seen from the results of in vitro tests on rats in the laboratory. Acute exposure to ammonium salts has an LD_{50} (lethal dose) value of 350–750 mg/kg body weight (WHO, 1996). This study aims to examine the toxicity level of ammonia as a fumigant for controlling *C. cynocephalus*.

MATERIALS AND METHODS

Research Site. The research was conducted at The Southeast Asian Regional Center for Tropical Biology (SEAMEO BIOTROP) Entomology Laboratory, Bogor.

Preparation of Test Insects. In this study, *C. cynocephalus* was used as a test insect. The termite specimens were obtained from the Forestry Research Center, Bogor City, Ministry of Forestry. The termites were then acclimatized for 3–5 days in a plastic container with a size of 40 × 20 × 30 cm, containing a block of sengon wood measuring 15 × 10 × 10 cm, and were maintained in the SEAMEO BIOTROP Entomology Laboratory at room temperature.

Preparation of the Test Wood. The test wood used in the study was dry sengon wood obtained from The Forestry Faculty Workshop of IPB. The wood was crushed and air-dried to a moisture content of 20%. The test wood had dimensions of length × width × height of 30 × 10 × 5 cm, 30 × 10 × 3 cm, and 30 × 10 × 1 cm. Each piece of test wood was cut into 2 equal lengths and then drilled to a depth of 10 cm with a drill hole diameter of 0.5 cm. As a result, three levels of wood thickness were obtained, namely 2.25, 1.25, and 0.25 cm. The test wood used in the experiment is shown in Figure 1. The thickness of the wood is calculated based on the formula below.

$$\text{Wood thickness} = \frac{T - D}{2}$$

T = Test wood height
D = Bor diameter

Preparation of Fumigation Chamber. The fumigation chamber frame was composed of a series

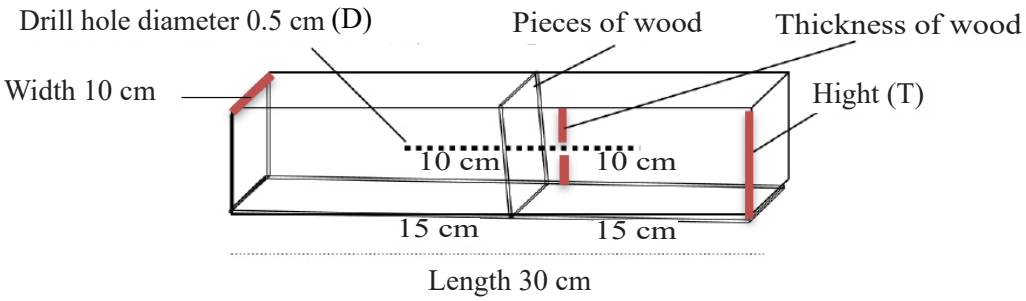

Figure 1. Tested wood with illustration different size in the investigation potential of ammonia as fumigant.

of 1-inch diameter PVC pipes. The fumigation room is approximately 1 m^3 in size. The fumigation space frame is then enclosed using a fumigation plastic sheet. After the fumigation chamber was formed, a sand snake was installed around the fumigation chamber to prevent gas leakage (Figure 2).

Stage of Fumigation. One hundred *C. cynocephalus* termites were infested into the holes of two pieces of tested wood, following the thickness level of the wood. The two pieces of wood were then merged using black duct tape, and a transparent duct tape was applied to prevent the fumigant from entering through the gaps between the two pieces of wood. The tested wood was then placed in the fumigation chamber.

Fumigation. According to the wood thickness level, 100 individuals of *C. cynocephalus* were infested into the holes on the test pieces of wood. The test wood was reconnected using black duct tape first to keep the wood from separating, and then taped again with clear tape to ensure that the fumigant did not enter through the gaps in the pieces of wood. The prepared test wood was placed in the fumigation chamber.

The ammonia fumigant used was in the form of ammonium hydroxide (NH_4OH). The dose of the ammonia fumigant was calculated in ml NH_4OH/m^3. NH_4OH was put into a measuring cup according to the treatment dosage and then placed in the fumigation room. The fumigation room was closed and ensured to be gas-tight, and a warning sign was posted.

Fumigation was carried out by evaporating NH_4OH in the fumigation chamber until the exposure time was reached. Once the exposure time was reached, the release (aeration) of ammonia gas was performed. Aeration is the process of removing residual fumigant from the fumigation chamber to a safe threshold level. This was done by opening the plastic on one side of the fumigation chamber after ensuring that the environment is safe for fumigation, with the help of a blower and air exhaust trunk.

Ammonia toxicity test for 2.25 cm thickness was carried out with 3 dose levels (0, 700, and 3800 mL NH_4OH/m^3) and 4 time levels (4, 6, 8 and 10 hours of exposure). Toxicity testing at 1.25 cm thickness was carried out at 6 dose levels (0, 200, 378, 587, 1732, 4188 mL NH_4OH/m^3) for 4 hours of exposure. The ammonia toxicity test at 0.25 cm thickness was carried out at 6 dose levels (0, 13.5, 31.5, 53.5, 211.5, and 653.5 mL NH_4OH/m^3) for 4 hours of exposure. Each treatment was carried out with 4 repetitions. Toxicity parameters were analyzed using probit analysis (Finney, 1971) with the Polo Plus program (Robertson et al., 2003) to determine Lethal Doses (LD_{50} and LD_{90}) with a 95%

Figure 2. Preparation of fumigation chamber. A. Stringing the PVC pipe; B. Installation of fumigation plastic sheet; C. Laying and arrangement of wood inside the fumigation chamber; D. Fumigation process.

confidence interval.

The observation of mortality due to toxicity in test insects *C. cynocephalus* was conducted under a microscope 24 hours after fumigation. The dead insects did not move and changed color to dark brown.

RESULTS AND DISCUSSION

Ammonia Toxicity as a Fumigant. The results showed that based on probit analysis, the toxicity of ammonia at a thickness of 2.25 cm was 3263 mL/m³ for LD_{50} NH_4OH and 22,781 mL/m³ for LD_{90}. The toxicity of ammonia at a thickness of 1.25 cm was 541.59 mL/m³ for LD_{50} NH_4OH and 1432.12 mL/m³ for LD_{90}. The toxicity of ammonia at a thickness of 0.25 cm was 67.67 mL/m³ for LD_{50} NH_4OH and 241.14 mL/m³ for LD_{90} (Table 1).

The killing power of the ammonia fumigant at different thicknesses produces varying levels of toxicity, which occurs due to sorption (fumigant penetration rate). Thicker wood requires higher doses to kill *C. cynocephalus*, whereas thinner wood requires lower doses (Darmawan et al., 2011). Insects are more tolerant of ammonia than other animal groups. Termites show acute tolerance to ammonia gas concentrations (Weihrauch et al., 2012). Besides being able to kill insects, ammonia can also maintain the quality of wood (Zhang et al., 2021).

The vapor pressure resulting from the application of hot ammonia fumigant will enter the wood through the cavities around the cellulose and increase the distance between the wood molecules (Zhang et al., 2021). This allows the ammonia to reach *C. cynocephalus*, which then damages the exoskeleton. The ammonia fumigant also inhibits the rate of metabolism by disrupting the acid-base balance in the insect's body, thereby reducing sensitivity to insulin, resulting in death. Ammonia can affect metabolism by altering the acid-base balance in the body, interfering with glucose tolerance, and reducing tissue sensitivity to insulin (WHO, 1996).

Ammonium hydroxide (NH_4OH) is liquid ammonia with a boiling point of -33,5 ºC and melting point -77 ºC, which has a poisoning effect in the gas phase (Appl, 1999). Based on this effect, ammonia fumigant is classified as a narcotic, meaning it can cause narcosis or loss of consciousness. The toxicity of ammonia is largely determined by its physical nature, as it is highly volatile and lighter than air, thus easily penetrating the cuticle and disrupting the metabolic rate of insects. Apart from being toxic to insects, ammonia can also control the fungi *Penicillium digitatum* and *Penicillium italicum* in post-harvest handling of citrus fruits (Herrero et al., 2010).

According to Prijono (2015), poisoning by a fumigant is caused not by the presence of a unique chemical structure in the fumigant molecule, but due to the physical presence of the fumigant molecule in the lipophilic part of the cell, known as the biophase (which plays an important role in the continuity of cell function). Fumigant is more of a physical poison than a chemical poison. Ammonia can affect metabolism by altering the acid-base balance in the body, interfering with glucose tolerance, and reducing tissue sensitivity to insulin (WHO, 1996).

The toxic nature of ammonia for living things can be seen from the results of in vitro tests on rats in the laboratory. Acute exposure to ammonium salts has an LD_{50} value of 350–750 mg/kg body weight. A single dose of 200–500 mg/kg body weight for various types of ammonium salts results in pulmonary edema, nervous system dysfunction, and kidney damage. Doses of 0.9% ammonium chloride (approximately 290 mg ammonia/kg body weight per day) in drinking water resulted in fetal growth restriction in pregnant rats (WHO, 1996). Exposure to 8 g/body weight of ammonia for 24 hours in marine animals such as *Sepia pharaonis* can result in death (Peng et al., 2017).

Table 1. Parameter of ammonia toxicity to the mortality of *C. cynocephalus* at 4 hours of exposure with different wood thickness

Thickness (cm)	*a*± SE	*b* ± SE	LD_{50} (CI 95%) (mL/m³)	LD_{90} (CI 95%) (mL/m³)
2.25	0.34 ± 0.63	1.16 ± 0.19	3263 (1866 – 13,777)	22,781 (7672–40,266)
1.25	-3.30 ± 0.48	3.04 ± 0.18	541.59 (482.59–611.85)	1431.12 (1185.78–1182.87)
0.25	1.25 ± 0.24	2.23 ± 0.13	67.67 (52.11 – 88.79)	241.140 (167.90–420.90)

a: Intercept of probit regression line; *b*: slope of probit regression line; SE: standard errors; CI: confidence interval

Toxicity of ammonia fumigant on wood with a thickness of 2.25 cm and exposure time of 4, 6, 8 and 10 hours resulted in doses that caused mortality of ≥ 50%. Therefore, a probit analysis was conducted to determine the LD_{50} and LD_{90} values of NH_4OH. The results of the probit analysis showed that exposure to ammonia fumigant had a toxic effect on *C. cynocephalus* with LD_{50} and LD_{90} NH_4OH values (Table 2).

Time is one of the main factors that must be considered during fumigation. Based on the results of probit analysis, the LD_{50} and LD_{90} values showed that the longer the exposure time, the lower the LD_{50} and LD_{90} NH_4OH values. This indicates that the longer the exposure time, the lower the dose of fumigant required. Aciar (1998) stated that time has an effect on the toxicity of a fumigant. The longer the fumigation exposure time, the higher the toxicity, and the lower the number of fumigant doses used. Ammonia fumigant has a much shorter exposure time when compared to methyl bromide and sulfuryl fluoride fumigants (Alfian et al., 2016).

The fumigants methyl bromide and sulfuryl fluoride have relatively short exposure times, while phosphine has a long exposure time and cannot be used for wood packaging (Agricultural Quarantine Agency, 2007b). Methyl bromide, used for the treatment of quarantine measures on wooden packaging, has an exposure time of 24 hours at a dose of 48 g/m³ (Agricultural Quarantine Agency, 2007a). Sulfuryl fluoride has an exposure time of 18 hours with a dose of 30 g/m³ on wood with a thickness of 10 cm (Alfian, 2015). Fumigation using phosphine requires a long exposure time between 7 to 20 days, with a dose of 1.5 g/m³ applicable only for food commodities (Aciar, 1998). The effectiveness of insecticides in killing the test organisms is usually expressed in a more specific quantity, namely the LD_{50}. Effectiveness in killing is usually referred to as toxicity (Dadang & Prijono 2008).

CONCLUSION

Fumigation using ammonia was toxic to *C. cynocephalus* in sengon wood with a thickness of 2.25 cm, with an LD_{50} value of 3263 mL NH_4OH/m^3 and an LD_{90} of 22,781 mL NH_4OH/m^3 for 4 hours of exposure. For the same length of exposure (4 hours), the LD_{50} and LD_{90} values for wood thickness of 1.25 cm were 541.59 mL NH_4OH/m^3 and 1432.12 mL NH_4OH/m^3, respectively. For wood thickness of 0.25 cm, the LD_{50} and LD90 values were 67.67 mL NH_4OH/m^3 and 241.14 mL NH4OH/m³, respectively. At a wood thickness of 2.25 cm, the LD_{50} and LD_{90} values decreased when the exposure time was longer. Specifically, the LD_{50} values were 2149 mL NH_4OH/m^3 for 6 hours of exposure and 1373 mL NH_4OH/m^3 for 8 hours of exposure. Similarly, the LD_{90} values decreased to 10,722 mL NH_4OH/m^3 for 6 hours of exposure and 8705 mL NH_4OH/m^3 for 8 hours of exposure.

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Table 2. Parameter of ammonia toxicity to the mortality of *C. cynocephalus* with 2.25 of wood thickness at different length of exposure

Exposure Time (hours)	*a*± SE	*b* ± SE	LD_{50} (CI 95%) (mL/m³)	LD_{90} (CI 95%) (mL/m³)
4	0.34 ± 0.63	1.16 ± 0.19	3263 (1866–13,777)	22,781 (7672–40,266)
6	-1.12 ± 0.65	1.84 ± 0.19	2149 (1144–4937)	10,722 (4753–31,240)
8	0.14 ± 0.59	1.59 ± 0.18	1373 (683–2405)	8705 (4085–11,706)
10	-	-	-	-

a: Intercept of probit regression line; *b*: slope of probit regression line; SE: standard errors; CI: confidence interval; - : could not be analyzed using probit due to 100% of mortality.

AUTHORS' CONTRIBUTIONS

AMI caried out the field and laboratory analyzed data, and wrote the manuscript. ISH and P designed this study and contributed to the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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