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SURVIVAL OF SUBTERRANEAN TERMITE COLONY FRAGMENTS *COPTOTERMES CURVIGNATHUS* HOLMGREN (ISOPTERA: RHINOTERMITIDAE) IN LABORATORY MAINTENANCE

Daya Tahan Hidup Fragmen Koloni Rayap Tanah Coptotermes curvignathus Holmgren (Isoptera: Rhinotermitidae) dalam Pemeliharaan di Laboratorium

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ABSTRACT

Information about colony fragments survival of the termite species *Coptotermes curvignathus* Holmgren in the laboratory is very limited. This study was conducted to determine colony fragments survival of the subterranean termite *C. curvignathus* Holmgren in laboratory maintenance. The test media used the Termitarium PSIH-IPB: 1999 as a glass vessel containing 4 kg of a mixture of sand and clay with 4 proportions and 4 different pH determined as treatments that would be studied for their effects on termite survival. The results showed that the proportion of the sand:clay mixture affected the survival and feeding preferences of the subterranean termite *C. curvignathus* Holmgren. The highest percentage of termites that survived was found in the termitarium containing a mixture of sand:clay 50:50. The highest percentage of bait wood weight loss occurred in the test media containing a mixture of sand: clay 50:50. The weight loss of bait wood is directly proportional to the termites survival.

Keywords: *Coptotermes curvignathus*; survival; clay; sand; subterranean termite; termitarium

ABSTRAK

Informasi tentang daya tahan hidup koloni atau fragmen koloni spesies rayap *Coptotermes curvignathus* Holmgren di laboratorium sangat terbatas. Penelitian ini dilakukan untuk mengetahui daya tahan hidup fragmen koloni rayap tanah *C. curvignathus* Holmgren dalam pemeliharaan di laboratorium. Media pengujian menggunakan Termitarium PSIH-IPB: 1999 berupa bejana gelas yang berisi 4 kg campuran pasir dan liat dengan 4 proporsi serta 4 pH yang berbeda ditentukan sebagai perlakuan yang akan ditelaah pengaruhnya terhadap daya hidup rayap. Hasil penelitian menunjukkan bahwa proporsi campuran pasir:liat berpengaruh terhadap daya hidup dan preferensi makan rayap tanah *C. curvignathus* Holmgren. Persentase rayap yang bertahan hidup paling tinggi terdapat pada termitarium berisi campuran pasir:liat 50:50. Persentase kehilangan berat kayu umpan tertinggi terjadi pada media pengujian berisi campuran pasir:liat 50:50. Kehilangan berat kayu umpan berbanding lurus dengan daya tahan hidup rayap.

Kata kunci: *Coptotermes curvignathus*; daya tahan hidup; liat; pasir; rayap tanah; termitarium

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A. INTRODUCTION

Over the past thirty years, experience has shown that termites are the most crucial wood-building and damaging organisms in Indonesia. The economic loss caused by the insect's attack on various lignocellulosic materials in buildings in Indonesia reaches IDR 8.67 trillion per year (Nandika 2015). Globally, of the approximately 2600 known termite species in the world (Kambhampati & Eggleton 2002), 80 species are recognized as very important wood destroyers, especially species of the genus Coptotermes (Kuswanto *et al.* 2015; Nanda *et al.* 2018). The damage caused by the attack of termite species of this genus occurs not only in building construction such as door and window frames, poles, and roof structures, but also in building contents, such as furniture, archives, and other valuables. The damage not only occurs in simple houses, but also in high-rise commercial buildings such as hotels, offices, shopping centers, museums, and others (Nandika *et al.* 2015). Rilatupa (2007), for example, reported that subterranean termites *Coptotermes curvignathus* Holmgren (Isoptera: Rhinotermitidae) were found attacking various building components on the 32nd floor of an apartment in South Jakarta. Besides its ability to attack building structures vertically, *C. curvignathus* subterranean termite colonies have an enormous population size, reaching 1.7 million individuals with a home range of 480 m^2 (Nandika 2000).

Given their ability to attack wood and buildings, along with their high population size and wide range, almost all standard methods for testing wood resistance to subterranean termites or for testing the efficacy of wood preservatives and termiticides against subterranean termites developed by various countries include certain species of *Coptotermes* spp. as *testing targeted ins*ects. In the Indonesian National Standard (SNI) 7207: 2014, for example, for testing the resistance of wood to subterranean termites or for testing the efficacy of wood preservatives against subterranean termites, the subterranean termite *C. curvignathus* is specified as the testing target insect. Likewise, the *Japanese Industrial Standard* (JIS) K 1571-2004 and the *American Society for Testing and Materials* (ASTM) D 3345-08 each list the genus Coptotermes as the target insect, precisely in the JIS standard lists *C. formosanus* and in the ASTM Standard lists *Coptotermes* spp. Arinana *et al.* (2012) reported that SNI 7207: 2014 and JIS K 1571-2004 show adequate reliability as standard methods for testing wood resistance to subterranean termites laboratory. In addition, ASTM D 3345-08 has also been widely used as a standard method for testing wood resistance to *Coptotermes* spp. termites (Dahali *et al.* 2020; Huaxu *et al.* 2021).

To apply the standard methods mentioned above, even for research on various aspects of subterranean termite biology and ecology, the availability of *laboratory-reared termite* cultures of *Coptotermes* spp. is essential. Concerning the process of testing the efficacy of wood preservatives and research on various bio-ecological aspects of wood-destroying termites in Indonesia, the availability of *C. curvignathus* subterranean termite cultures is a major pre-requisite. However, until now, no comprehensive scientific information has been available on the survival of subterranean termites C. *curvignathus* in the laboratory rearing process and its relation to the micro-environmental conditions in the laboratory. Even reliable and tested standard methods for rearing or breeding these subterranean termite species still need to be developed, including the media characteristics that are most suitable for supporting the life of these termite species in laboratory conditions for a longer time. Until now, the test media used in laboratory-scale test standards (SNI 7207:2014 and ASTM D3345-08) is sand. Meanwhile, according to Lee & Wood (1971), termites generally dislike sandy soil and prefer soil types that contain a lot of clay. Meanwhile, according to Robinson (1996), subterranean termite burrow networks are more easily built by termite colonies in soils with a balanced sand, dust, and clay composition than in soils with too high clay or sand content. In addition to soil texture, it is also essential to condition the pH of the soil used as testing media so that it can optimally support termite life in laboratory maintenance. However, until now, there has been no standard that demands or requires a certain level of soil pH in testing media related to subterranean termites *Coptotermes* spp. in the laboratory. While some research results (Arinana *et al.* 2019; Arinana *et al.* 2020a; Arinana *et al.* 2020b) show that *Coptotermes* spp. subterranean termites can live at pH 4.23 to 8.47. Especially in testing *Coptotermes* spp. subterranean termites in the laboratory, several studies have also been conducted using soil media with pH 5 to 5.49 (Himmi *et al.* 2013; Bhatta & Henderson 2016).

In connection with the above, it is necessary to conduct research on the survival of colony fragments of subterranean termites *C. curvignathus* in the laboratory's maintenance process using different proportions of clay sand and pH.

B. METHODS

Materials and Tools

The materials used were pine wood, sand, clay, subterranean termites *C. curvignathus*. The equipment used were glass vessels (bottom diameter 30 cm, top hole diameter 15 cm, height 35 cm), Keyence VHX 6000 digital microscope, Lux Meter type LX1010B, Digital Temperature Humidity Data Recording Logger Meter brand YUWEN type DWL-20, pH meter (4 in 1 Soil Survey Instrument), air conditioner, oven, desiccator, and digital balance.

Procedures

1. Procurement of Subterranean Termite Colony Fragments

Colony fragments of subterranean termites *C. curvignathus* were collected from three spots around the Experimental Forest, Research and Development Agency for Environment and Forestry, Ministry of Environment and Forestry in Dramaga, Bogor, respectively at coordinates 33.479'S, 45.241'E; 33.480'S, 45.241'E; and 33.479'S, 45.242'E using the *baiting* method developed by Nandika (2015). In each spot, 48 pine wood boards measuring 60x10x2 cm (12±3% moisture content) as bait wood were stacked in a row in the width direction above the ground (6 boards in the width direction, 8 boards in the height direction). The bait wood was covered with a black plastic sheet and left in each spot for 1 month (Figure 1). After the baiting period ended, soldier caste specimens from each spot were taken and placed in a collection bottle containing 70% alcohol, then identified according to the key to Termite Identification according to Tho (1992) and Ahmad (1958; 1965). The termite soldier caste specimens were also photographed using a Keyence VHX 6000 digital microscope. If the termite specimens were identified as *C. curvignathus*, each pile of bait wood and the termite colony fragments trapped in it were transported to the laboratory for 30 days of *conditioning*.

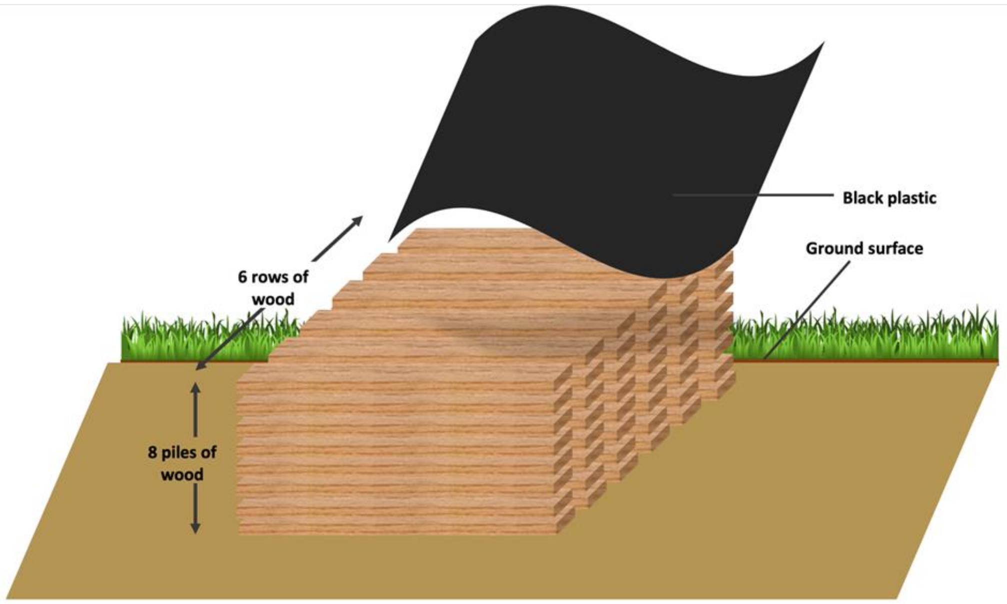

Figure 1. Pine bait wood pile at subterranean termite feeding site

2. Termitarium Preparation

The terrarium was made following the PSIH-IPB terrarium specification: 1999 developed by Diba & Nandika (1999), in the form of a glass vessel with a bottom diameter of 26 cm, a top hole diameter of 17.5 cm, and a height of 26 cm, in the center of which there is a hole (1 cm diameter) filled/passed by a 10 cm long stove wick. The vessel was filled with a mixture of sand and clay, as much as 4 kg, which had been sterilized in an autoclave at 121 ºC for 1 hour (Mahmood *et al.* 2014). The sand and clay mixture that was put into the thermitarium consisted of four different proportions, namely 100:0, 75:25, 50:50, and 25:75 (w/w), with pH 6.5, 6, 5.5, and 5, respectively. For each proportion of the sand and clay mixture, three thermitariums were prepared as replicates (Table 1). Thus the total of thermitariums with 4 different proportions of sand and clay mixture and 3 replications were 12 thermitariums.

Table 1. The proportion of sand and clay used as testing media

Proportion of sand : clay	pH	Repetition
100 : 0	6.5	3 times
75 : 25	6	3 times
50 : 50	5.5	3 times
25 : 75	5	3 times

The moisture content of the sand and clay mixture was 7% below the *water-holding capacity* (ASTM D3345 2008; SNI 7204 2014). On the surface of the sand and clay mixture, one piece of pine wood (*Pinus merkusii* Jungh et de Vries) that had been *steamed* for 5 hours, 5x5x2 cm in size and 12±3% moisture content was placed as a food source for termites (bait wood) (Arinana *et al.* 2012; Arinana *et al.* 2020a). The vessel was placed in a plastic tray (30 cm diameter) with water at the bottom, such that part of the stove wick from the bottom of the thermitarium dangled into the water at the bottom of the tray. The wick serves as a conduit for moisture into the sand and clay mixture in the vessel. Fragments of field-fed subterranean termite colonies of *C. curvignathus* consisting of 1800 workers and 200 soldiers were placed in the vessel (Figure 2).

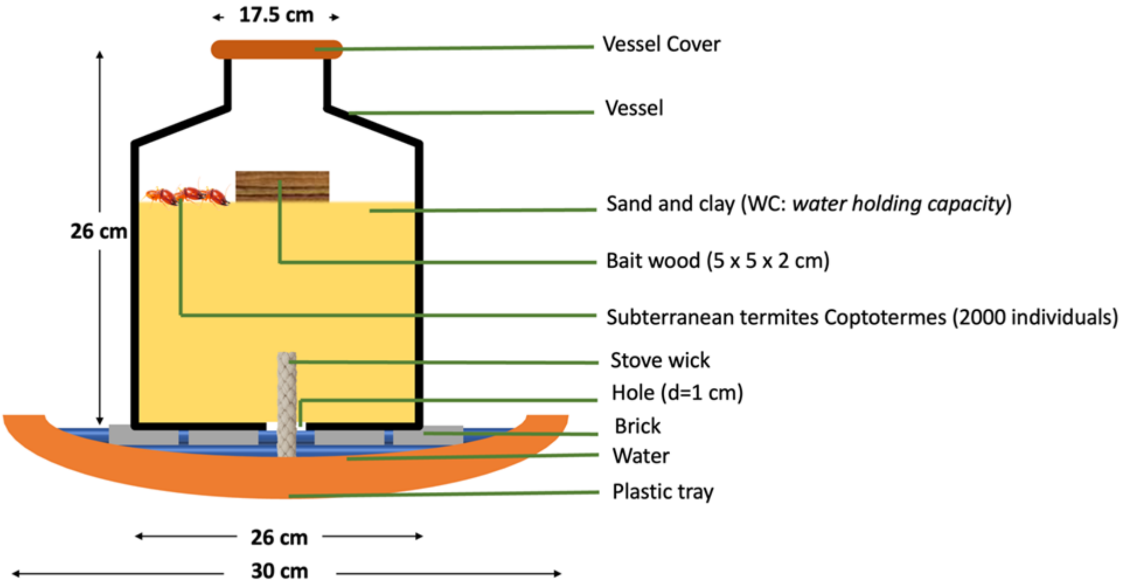

Figure 2. Glass vessel as testing media container

3. Observation and Data Collection

The entire thermitarium was kept in a dark room (morning temperature 25.3 ºC; afternoon 25.9 ºC; evening 25.6 ºC, morning air humidity 88.3%; afternoon 88%; evening 88.5%, light intensity 003 lux) for three months. The air temperature and humidity in the room were also measured using a *Digital Temperature Humidity Data Recording Logger Meter,* while the light intensity of the room used a Lux Meter type LX1010B (SNI 7062: 2019). After three months, each thermitarium was dismantled, and the percentage of termite *survival* and weight loss of bait wood were calculated, each using the following formula.

The following formula expresses the percentage of termites that survive (survival):

$$\text{Termite Survival Percentage (\%)} = N2/N1 \times 100$$

Where, N1 was the number of termites at the beginning of the test in gram (g) and N2 was the number of termites at the end of the test in gram (g).

The following formula expresses the percentage weight loss of feed wood:

$$\text{Weight Loss (\%)} = (W1-W2)/W1 \times 100$$

Where, W1 was the weight of bait wood at the beginning of observation in gram (g) and W2 was the weight of bait wood at the end of observation in gram (g).

Data Analysis

Descriptive statistical analysis was used to present information in tables and graphs. The research was arranged in a factorial (1x4) completely randomized design (CRD). The single factor was the proportion of sand and clay with 4 levels (100:0, 75:25, 50:50, and 25:75). The data obtained were analyzed by variance analysis (ANOVA) using the following mathematical model:

$$Yij = \mu + \alpha i + \varepsilon ij$$

Where, Yij was the observation in the i-th treatment of the j-th replication, μ was the generalized mean value, αi was the effect of the factor of the proportion of sand and clay at level i, and εij was the random effect on the i-th treatment and j-th replication.

C. RESULTS AND DISCUSSION

Morphology of Subterranean Termites

The subterranean termite species used as the test insect in this study is Coptotermes *curvignathus* (Figure 3). C. *curvignathus* is characterized by a flat to broadly rounded head shape, yellow to orange head color, and antennae of 14-16 segments (Ahmad 1965; Tho 1992). Measurement results from the soldier caste show that C. *curvignathus* termites have a head length of 0.95 mm, head width of 0.81 mm, left mandible length of 0.54 mm, right mandible length of 0.58 mm, pronotum length of 0.31 mm, pronotum width of 0.6 mm, posmentum length of 0.7 mm, and posmentum width of 0.17 mm (Figure 3). In addition, one of the characteristics of C. *curvignathus* termites is the shape of the posmentum, which is narrowed in the middle.

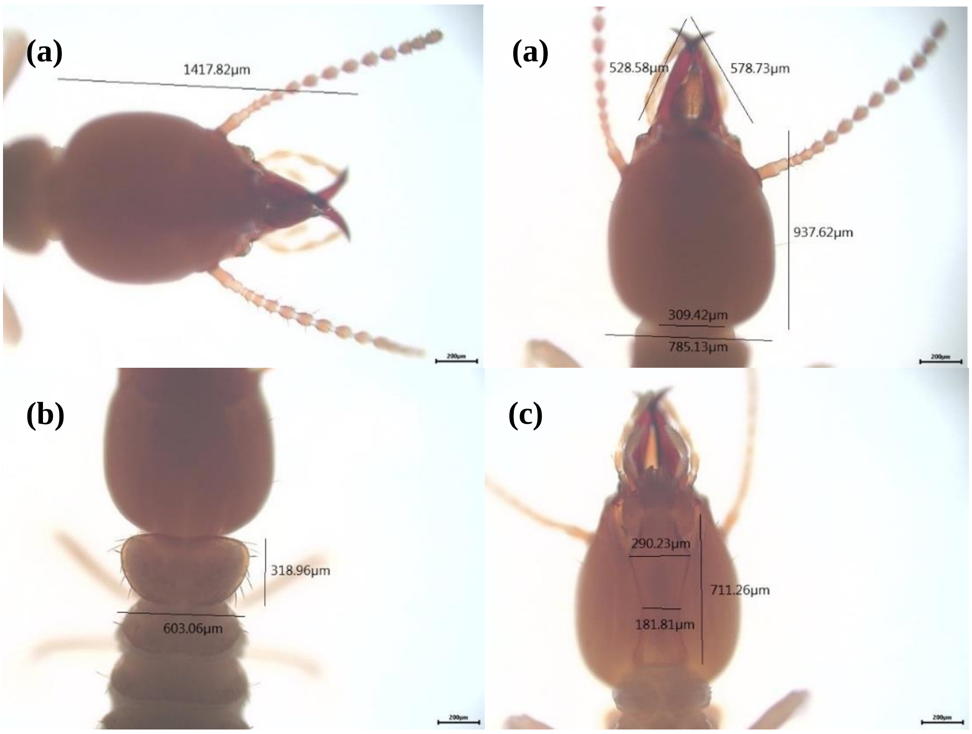

Figure 3. Morphology of the *Coptotermes curvignathus* soldier caste: head (a), pronotum (b), and posmentum (c)

Termitarium Microclimate

The results showed that the average moisture content of the sand and clay mixture in the thermitarium was± 40.25%. The results of the analysis show that the more clay content of the sand:clay mixture media, the less the ability to hold water in the sand:clay mixture (Figure 4).

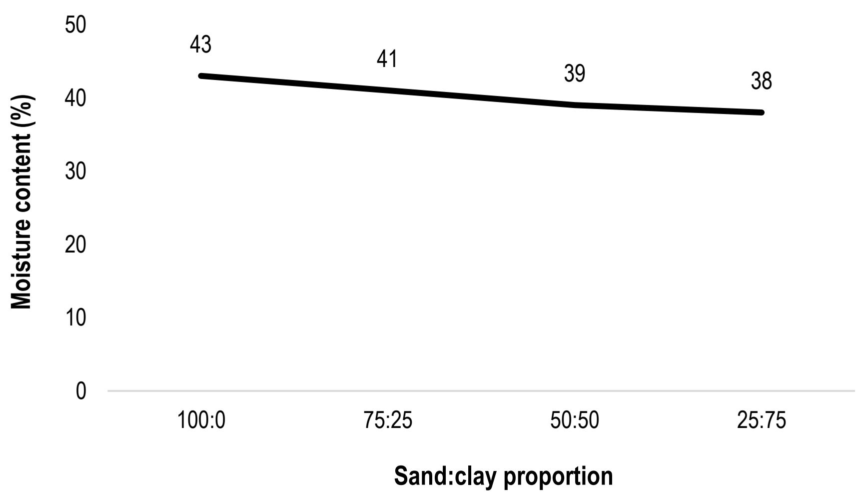

Figure 4. Moisture content of sand:clay mixture

The results showed that the average pH of the sand:clay mixture ranged from 5 to 6.5. The highest pH was found in the 100:0 sand:clay mixture, followed by 75:25, 50:50, and 25:75 (Figure 5). Arinana *et al.* (2019); Arinana *et al.* (2020a); Arinana *et al.* (2020b) showed that subterranean termites *Coptotermes* spp. can live at pH 4.23 to 8.47 in field tests. The results also showed that the more clay content in the soil media used, the more acidic the soil media.

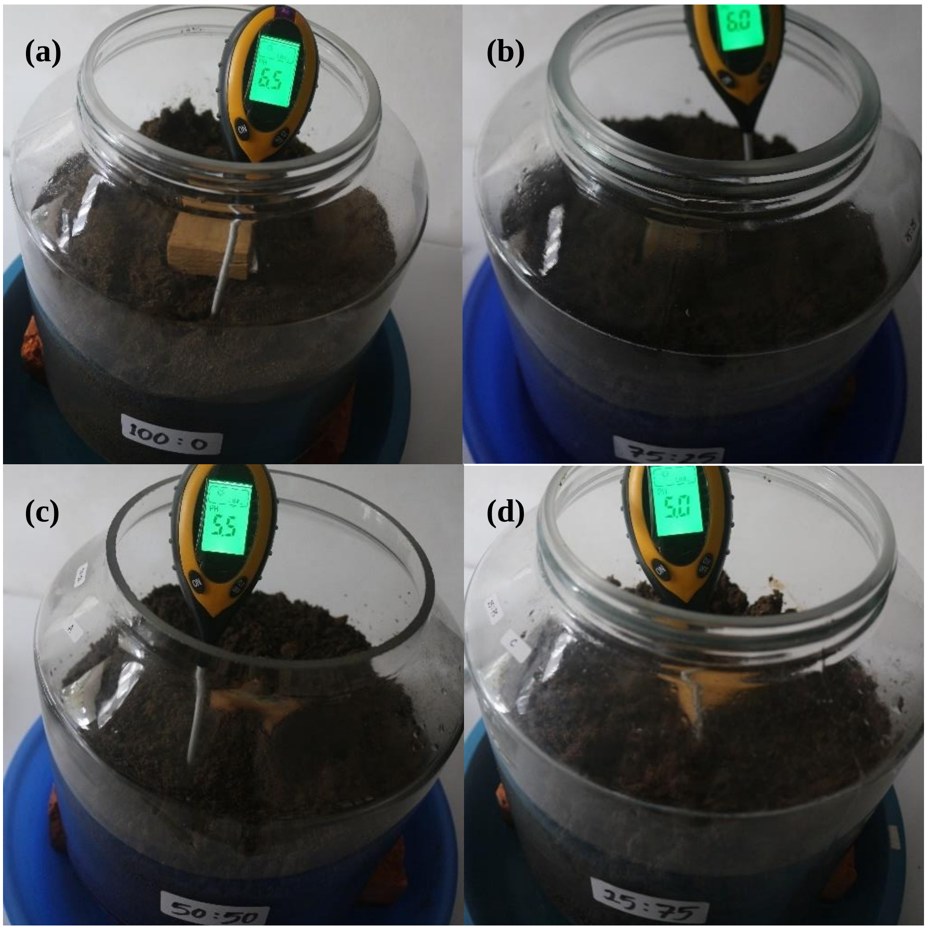

Figure 5. pH of sand and clay mixtures 100:0 (a), 75:25 (b), 50:50 (c), and 25:75 (d) in the thermitarium

The results show that the light intensity in the test dark room was ± 3 lux (Figure 6). This result meets the requirements for thermitarium storage. This is in line with what Lindsey (2010) reported, which is that termite activity and food consumption are higher in the dark room than in the lighted room. Meanwhile, Lee & Wood (1971) suggested that termites often stay underground or in the nest when the sun shines directly on the ground surface.

Figure 6. Light intensity in dark room testing

Temperature and humidity also significantly affect the behavior of subterranean termites. The results showed that the highest air temperature in the testing room was± 26.3° C. Meanwhile, the air humidity in the testing room was ± 88.5% (Figure 7). Arinana *et al.* (2019) reported that the minimum temperature around the nests of subterranean termites from the Rhinitermitidae and Termitidae families in DKI Jakarta Province was 21.9 ºC - 26.1 ºC and the maximum temperature was 35.3 ºC - 38.8 ºC. Meanwhile, Zukowski & Su (2019) suggested that the optimum RH that supports the life of subterranean termites *C. formosanus* is 75-81%.

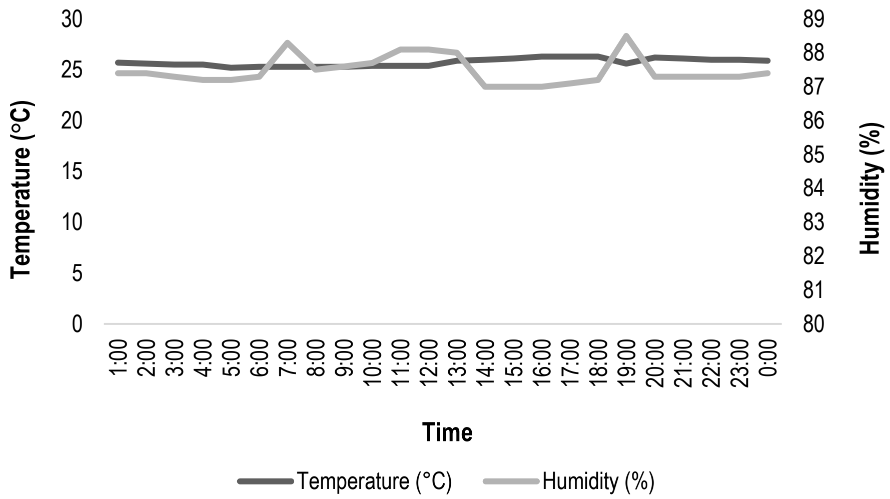

Figure 7. Temperature and humidity in the test darkroom

Survival of Termites

The results showed that the average percentage of surviving termites after three months of testing ranged from 2.95% to 26.00%. The highest percentage of surviving termites was in the 50:50 sand:clay mixture, followed by the 75:25, 100:0, and 25:75 sand:clay mixtures (Table 2). These results align with those stated by Lee & Wood (1971) and Thuyne & Verrecchia (2021) that termites generally prefer clay-containing soil to sandy soil. Meanwhile, Cornelius & Osbrink (2010) also reported that termite survival is better in soils with sand and clay content. Termite survival can also be seen from the ability of termites to make burrows or the ability of termites to reach their food sources. Robinson (1996) suggests that subterranean termites more easily build termite burrow networks with a balanced soil texture compared to more sand or clay content.

Table 2. Percentage of termite survival

Sand:clay proportion	Number of termites at the beginning of the test (g)	Number of termites at the end of the test (g)	Percentage of termite survival (%)
100:0	6.53	0.23	3.48[b]
75:25	6.53	1.12	17.21[c]
50:50	6.53	1.70	26.00[d]
25:75	6.53	0.19	2.95[a]

The analysis of variance showed that the proportion of sand:clay significantly affected the survival of colony fragments of subterranean termites *C. curvignathus* in the thermitarium during the three months of testing (5% absolute level). Subekti *et al.* (2018) state that soil texture affects the survival of subterranean termites in buildings. In addition, several essential factors also affect termite survival, especially in the laboratory, such as temperature, air humidity, and light in the surrounding environment (Arinana *et al.* 2016).

Feed Wood Weight Loss

The results showed that the average weight loss of bait wood after three months of exposure to colony fragments of subterranean termites *C. curvignathus* ranged from 3.71% to 10.46%. The highest weight loss of bait wood occurred in the test media containing 50:50 sand:clay mixture, followed by 75:25, 100:0, and 25:75 sand:clay mixture (Figure 8). The analysis of variance showed that the proportion of sand:clay significantly affected the weight loss of bait wood after being exposed to colony fragments of subterranean termites *C. curvignathus* for three months in the laboratory (5% absolute level).

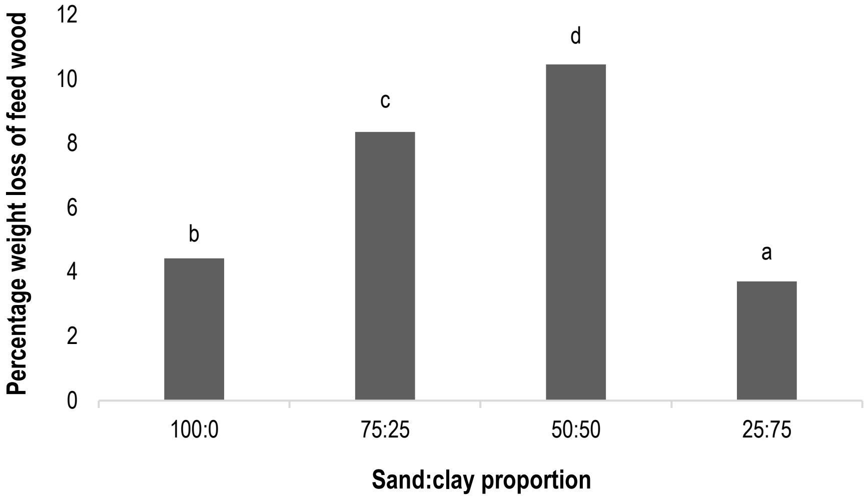

Figure 8. Weight loss of bait wood (letters a-d are the results of Duncan's test which shows significant differences in each treatment)

The weight loss of bait wood is directly proportional to the percentage of survival of termites, where the percentage of survival of termites affects the weight loss of bait wood. The highest percentage of termite survival produces the highest percentage of bait wood weight loss. This is influenced by the consumption of bait wood by the number of termites that can survive until the end of the test.

The low survival rate of termites leads to lower bait wood weight loss due to the large number of dead termites (Cornelius & Osbrink 2015; Febrianto *et al.* 2015). The results of the variance analysis further showed that the survival of colony fragments of subterranean termites *C. curvignathus* significantly affected the weight loss of bait wood after three months of laboratory testing (5% absolute level).

D. CONCLUSION

The proportion of sand and clay mixture affects the survival of subterranean termites *C. curvignathus* in the laboratory for three months of testing. The highest percentage of surviving termites was in the 50:50 sand:clay mixture, followed by 75:25, 100:0, and 25:75. A balanced proportion of sand and clay mixture is preferred by *C. curvignathus* subterranean termites compared to more sand or clay content. The sand and clay mixture proportion also affected the weight loss of bait wood exposed to *C. curvignathus* subterranean termites in the laboratory during the three-month test. The highest percentage of bait wood weight loss occurred in testing media containing a 50:50 sand:clay mixture, followed by 75:25, 100:0, and 25:75 sand:clay mixtures. The percentage of bait wood weight loss is directly proportional to the percentage of termite survival, where the percentage of termite survival affects the weight loss of bait wood.

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