Directional response of the subterranean termite *Coptotermes curvignathus* toward volatilized *Pinus merkusii* extract

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**Abstract.** Preliminary study on termite recognition for food source location was carried out by the subjection of volatile compounds emitted by Sumatran Pine (*Pinus merkusii* Jungh. & de Vriese). Food source recognition by termite was evaluated using a set-up model on the inverted Y-tube apparatus. The apparatus consisted of a release chamber, in which five workers had been released; an intersection chamber, in which termite movement would be evaluated for clockwise and counter-clockwise movements; and a pair of food chambers. Between the intersection chamber and food chambers, a tunnel with 5 cm length was divided into five scale ranges, in which each range was related to a preference score. Various set-up models had been prepared from these concentrations: 0%; 0.5%; 1%; 2%; 4%; 8% of the macerated extract of Sumatran Pine sapwood in 8 mm paper dish. Each set-up consisted of a pair of concentrations: low & high concentration, which was located on different food chambers. The results suggested that termite showed directional preference to a low concentration, even in a set-up model of 0% & 0.5% ($F= 16.65$, $P = 0.002$), which indicated that the extracts might have a repellent effect against termite. When the results were clustered based on termite’s movement, clockwise and counter-clockwise, significant preference for low concentration was observed in two and one set-up models, respectively. Behavioural observation showed that in most of the set-up models, the first food chamber explored by a termite would generate more preference scores, regardless of the concentrations.

1. **Introduction**

Subterranean termite *Coptotermes curvignathus* Holmgren is an endemic species of termite from South-East Asia that has been classified as an urban pest in the region [1]. It was known as the largest and most aggressive among the oriental *Coptotermes* spp. [2]. Genus *Coptotermes* takes up to 80-90% of total finding in infested premises in northern peninsular Malaysia, Thailand, and Singapore [3]. It was also suspected that similar incidence also exists in the other close area such as Indonesia. The high incidence rate of *Coptotermes* genus can be attributed to its linear reproductions that ensure its survival through small colony numbers and its capability to adapt in many different terrains and food sources [3–5].

Sumatran pine (*Pinus merkusii* Jungh. & de Vriese) is an evergreen tree categorized as softwood and has been found quite susceptible to termite attacks with mass loss ranging from 20-25% under three weeks of infestations [6–9]. Sumatran pine sapwood susceptibility to termite attacks makes it an ideal subject to be used as termite food when termite response is observed during food foraging.

Based on previous studies, the termite will use various clues in the environment to reach food at the most efficient way including termite choices in making gallery during foraging [10–12]. One of the clues that have been studied in relation to termite foraging is an olfactory signal, especially volatile
chemical compounds. Some studies showed that volatile chemical compounds could induce termite to have specific response whether gathering or avoiding particular object [13–16]. By using volatile chemical compounds extracted from known timber susceptible to termite attack such as Sumatran pine, it can give richer understanding toward termite response during food hunting period.

Therefore, the study aim is to observe termite directional response to double choices of concentrations of Sumatran pine sapwood macerated extract. The final preference choice, general termite movement response and also the intensity of extract concentration effect towards termite response will be observed.

2. Materials and methods

2.1. Wood and termite source

Sumatran pine (Pinus merkusii) was obtained from Yanlapa Research Forest (KHDTK Yanlapa) managed by Research Center for Forest Development - Indonesian Ministry of Environment and Forestry. The tree was 60 years old when cut down. The wood materials used was the sapwood area, known to be easily infested by termite when exposed. The termite colony was collected from Sumatran pine stumps from the same plantation. The entirety of termite colony was moved together with Sumatran pine stumps into a 50-liter plastic box and kept in T=28 °C with H=80% before bioassay was conducted.

2.2. Sample preparations

Agitated maceration was conducted as a means of extractions. Sapwood was shade dried till its moisture content reached 10-11% (d/w) and then granulated by disk milling (Hongtongfang®, Shandong, PRC) and sieved to have granulated sapwood (>60 mesh). As much as 35 g (d/w) of granulated sapwood was put in erlenmeyer flask and diluted with analysis-grade methanol (Merck, Darmstadt, Germany) until the volume reached 350 ml (w/v). The agitated maceration used incubator shaker (WIS-30, Daihan Scientific, Gangwon-do, ROK) with parameters as follows t=48 h, T=30 °C, and r=150rpm. The extract was filtered with filter paper (Whatman plc, USA) and the filtrate was evaporated.

The left-over residue was then freeze-dried to obtain the end product. The end product was diluted using methanol for analysis to make a stock solution of 8% concentration. Using micropipette (PIPETMAN classic, Glison, France) through a series of dilution, solution concentrations of 4%, 2%, 1%, and 0.5% was made and stored in the refrigerator for further usage.

2.3. Double choices bioassay

The bioassay was conducted using an inverted Y tube apparatus. In each of food chambers, an 8 mm paper dish (Advantec®, Japan) was pipetted in with 25 µL of extract in sets of concentrations pair (low & high concentration) as follows 0%|0.5%; 1%|2%; 2%|4%; 4%|8%. For each set-up, placement order of the higher concentration between the two food chambers was divided into two clusters (clockwise and counter-clockwise). For each run, five workers were released into release chamber, and recorded (Oppo A37, Dongguan, PRC) for 30 minutes. For each run, termite preference, response frequency and dominant preference were measured all together.

For each movement observed in connecting tunnel, a score was given when termite was at its closest locations to corresponding food chambers. Only termite’s movement that was commenced from intersection chamber can be given a score. The score was tied into five scaled range that divides the length (5 cm) of the connecting tunnel into 0 to 0.5 cm = 0.1; 0.5 to 2 cm = 0.2; 2 cm to 3.5 cm = 0.4; 3.5 cm to 5 cm = 0.6; >5cm and immediately returns = 0.8; and spends sometime in the food chamber = 1.00. The schematic is shown in figure 1.

2.4. Data analysis

2.4.1. Termite preference. Termite preference was measured by calculating a number of directional responses in each food chambers direction by plotting the distance traveled into sets of score that had been put forward before to build preference score for each food chamber. The distance traveled by termite was measured from the starting point (intersection chamber). Each total preference score from
one set-up model was analyzed using the unpaired t-test with three replicates for each clustered (clockwise and counter-clockwise). A significant result was indicated by P-value lesser than 5%.

Figure 1. Inverted Y Tube bioassay schematic. All measurements are in centimetre.

2.4.2. Response frequency. Response frequency (Rf) is a measurement of termite activity based on a number of observed directional response between two food chambers without the inclusion of weighted score. The Rf value is used to monitor the type of termite movement response under the effect of certain stimuli. There are two categories of termite movement response namely: approach (exploring connecting tunnel) and intrusion (exploring food chamber). For each movement response observed, a score of 1 was given regardless of termite position in the connecting tunnels or the food chamber. Total score from each set up was analyzed using unpaired t-test with three replicates for each clustered (clockwise and counter-clockwise). A significant result was indicated by P-value lesser than 5%.

2.4.3. Dominant preference. The measurement was intended to evaluate the impact of given stimuli (food extract) for termite against other interfering factors, in this case, suspected pheromone trail. The measurement is a binary success/failed assessment to determine which factors are dominant in ruling directional response. The first criteria (Cr-1) is “for every first explored food chamber in a set-up model; by the end of observation, the preference score will prevail in that exact food chamber” and if it is failed then, the second criteria is applied. The second criteria (Cr-2) is “food chamber consist of food extract that termite prefers will have higher preference score by the end of observation time”. The result will be counted.

3. Result

3.1. Termite preference
It is surprising that for general trend observed for both movement setting, low concentration yielded higher means of preference score compared to high concentration considering the volatilized extract was extracted from sapwood that has been classified as vulnerable of termite attack. Even though the general means of preference score favor low concentration, for most of the observation, the difference is not truly significant. Interestingly, model A-0|0.5 (clockwise setting) showed a significant difference
(F= 27.61, P = 0.0063) leaning to zero concentration compared to its 0.5% pair while for counter-clockwise setting at the same set-up model showed no significant preference. Preference score of low concentration in the A-0|0.5 model is also significantly different compared to other low concentrations at many others set up models. The preference score results of the set-up model can be seen in figure 2.

![Figure 2](image)

**Figure 2.** Termite preference between clockwise setting (A) and counter-clockwise setting (B). Different letters indicate a significant difference between the two values observed in a set-up model.

3.2. Response type frequency
The result on response type frequency can be summarized to have a similar result with termite preference especially between clockwise of termite preference and approach response type. The similarity is on the significant difference of response score for zero concentration compared to other low concentrations at many others set up models. Other than that, observed means during the experiment showed certain trend. Termite response under clockwise tended to decrease in approach response type toward a higher pair of concentrations while the opposite happened in the counter-clockwise group. On the other hand, the trend for intrusion response type is not significantly different between two movement clusters with means of counter-clockwise showing higher means all across set-up models. When the detail of response type was compared internally for each setups, a similar trend was seen except for counter-clockwise movement where a higher concentration have a higher score of approach response type compared to low concentration.

3.3. Dominant preference
Based on observation of timestamp when the first intrusion to food chamber happened and comparing the data to the final tally of preference score, it was found that for the first criteria, the clockwise movement was 93% fulfilled while counter-clockwise was 73% fulfilled. Comparing the failed data to second criteria resulted in 83.3% fulfilment. All the failed data that fulfilled second criteria had high concentrations as the first food chamber visited.

It is important to note that up to 25% of total observation showed termite picking high concentrations first over low concentrations in clockwise movement while the result for counter-clockwise was up to 40% even though preference test showed that termite preferred low concentrations.
Figure 3. Termite response frequency between approach response type (A) and intrusion response type (B). Different letters indicate a significant difference between the two values observed.

Figure 4. Termite dominant preference observed between criteria (A) and termite first chamber explored based on model setting (B).

4. Discussion
The combined result of termite preference, response type frequency, and dominant preference divided by movement cluster and different intensity of olfactory signal tells a comprehensive view towards termite response when exposed to a volatilized extract of the food source. It has come to our attention that the defining trait of termite preference most likely comes from the surrounding environmental conditions. Some past research has uncovered some cues in understanding termite response behaviour,
for example during foraging especially the actions after encountering various conditions such as physical objects, gradient of certain chemical compounds, or the appearance of other termite species whether alive or dead [8, 12, 17, 18]. In our observation, we observed different directional responses such as aversion to indifference when put in situation that requisite termite to move clockwise or counter-clockwise. The change of directional responses created through different placement of food source in respect to initial termite location is actually a new finding for us.

The data also suggest that the different type of movements not only affect termite directional responses but also affect the termite’s way of approaching the source of volatilized food extract especially in high total intensity of food extract. Termite generally shows an increasing level of reluctance for tunnel exploration (clockwise setting) to an increasing level of protracted movement in connecting tunnels (counter-clockwise setting). As of note, the total intensity is defined as the sum value of concentrations from both food chambers. On the other hand, the change of relative intensity between set-up models, which is high intensity divided by low intensity, gives us a common pattern of termite response towards volatilized food extract.

The pattern seems to insinuate that termite will significantly prefer food chamber without food extract than food chamber containing food extract. It is a surprising finding, not because of its repellent effect but because of how termite still avoids very little concentration of food extract. It was known that extractive material of wood such as Teak (Tectona grandis) which is less preferable could have a repellent effect because of its higher phenol concentration [8, 19, 20]. Therefore, the utilization of vulnerable wood like Sumatran pine as food extract can be justified.

Based on the observed result of past feeding experience [21] and the usage of Sumatran pine as past feeding before experiments, we expect that by decreasing the concentration of food extract, we can simulate the odour of Sumatran pine on the paper dish and achieve an arresting effect for termite to stay near the paper dish. Considering data at hands, a more in-depth study in a smaller range of concentration and longer time of observation is necessary. Furthermore, the data also gives some clarity for determining final preference for termite that seems to be dictated by pheromone trails strength left behind by pioneer termite when the pioneer termite returns to the entry point [22, 23].

It is known that pheromone trails are used as guiding signals to go outside or inside the nest [23], as marker toward a selected food source and also as a marker for nestmate identification [23, 24], so it is expected to give some interference in data analysis. Interestingly, the interference observed presents us with an interesting relationship between termite response to food source while under the influence of pheromone trails. The data suggest that while other termite initially follow pheromone trails left behind by pioneer termite, for some situation where a high concentration of food extract becomes the first chamber explored, almost a half of them develop aversion and choose low concentration chamber by the end of observation (Criteria-1 at figure 4). This finding indicates that termite may develop a dynamic sensing mechanism against volatilized chemical compounds, in this case, is between volatilized food extract and pheromone trails.

Previous studies have shown that termite moving velocity to a food source by following trails depends on quantities and concentration of pheromones [25–27]. It was also reported that certain actions such as foraging, recruitment, and marking food source, there would be a different component of chemicals used as pheromone constituents. Some chemicals can be in volatile form while some others have low volatility that can remain in place for a longer period, in some cases even more than a year [28]. Inferring from the balance of volatilized chemical compounds detected by termite, it seems that swing responses are dependent on quantities and pheromone concentration where low quantities and concentrations can induce aversion since the pheromone signal received by termite is overwhelmed by volatilized food extract.

In the end, the question about the process of decision making (aversion/fondness) of pioneer termite towards given food extract stimuli is still unclear. The interpretation from the collected data indicates that the role of movement clusters (clockwise/counter-clockwise) as the main cause to direct pioneer termite in foraging. This can be seen from the counter-clockwise setting that showed almost equal proportions in terms of first food extract reached regardless of concentrations available while the clockwise setting was shown to prefer low concentration. The possible working mechanism seems to be governed by the difference of termite pheromone trail constituents between the two different movement
clusters. Based on some reports that had been discussed previously, it is possible that certain constituent of pheromone trails is produced during counter-clockwise setting or differing of general concentration of pheromone which managed to give termite an edge to come nearing the high concentration of volatilized food extract well above the situation under the clockwise setting. A study is required to unravel the cause of observed swing response observed in treated termite.

5. Conclusion
Termite for some extent chooses to avoid volatilized extract from Sumatran pine sapwood with the significant result is shown between pairs containing zero concentrations. The different type of movement may affect pioneering response against volatilized extract that can yield aversion (clockwise) to indifference (counter-clockwise) behaviour by the end of observation. The development of late aversion behaviour in some observed experiments might be caused by the weaker signal of pheromone trails received by termite compared to volatilized food extract.

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