

**THE PROCESSING AND TRANSFERRING OF FOOD IN THE COLONY
OF *Odontotermes hainanensis* Light (Isoptera: Macrotermitinae)**

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ABSTRACT

Odontotermes hainanensis Light is known as a pest to structures, crops, dikes and dams in Vietnam that needs to be controlled. Currently, termite control using baits is considered a pre-eminent especially environmentally friendly measure. However, applying this method has been proven to be incapable to control *Odontotermes hainanensis* or other fungus-growing termites (the subfamily Macrotermitinae) due to their complex food processing. The research on food processing and the food distribution ability within the colony of this species was conducted in order to identify a scientific basis to further enhance the efficiency when treating them with baits. Results on the food processing of laboratory cultured colonies showed that this process took from 21 to 35 days with labor division in worker groups. Results on food transferring by food marking method in the field colonies showed that food was distributed by workers to most of the fungus garden cavities of the colonies. The proportion of cavities that fungus combs containing marking food reached from 92.1% to 100% in these studied nests. This is an essential scientific basis for the termite bait study on controlling the fungus-growing termites, particularly *Odontotermes hainanensis*.

Keywords: Food processing, food transferring, fungus-growing termites, *Odontotermes hainanensis*.

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INTRODUCTION

Odontotermes hainanensis Light is a major pest of dikes and dams (Nguyen Duc Kham, 1976; Vu Van Tuyen, 1982; Trinh Van Hanh, 2008; Ngo Truong Son, 2009), is harmful to construction works and relics (Nguyen Tan Vuong, 2007; Nguyen Thi My et al., 2014). It is, thus, needed to be controlled. At present, the controlled method by termite bait has advantages not only in terms of efficiency but also in environmental protection. In recent years, bait products containing active ingredients that inhibit the synthesis of chitin have been studied and developed such as hexaflumuron, bistrifluron, lufenuron, etc. These bait products are highly effective against lower termites, including *Coptotermes curvinagthus* Holmgren (Sajap et al., 2000), *Coptotermes travians* (Haviland) (Lee, 2002), *Coptotermes formosanus* Shiraki (Su et al., 1997; Rojas & Morales-Ramos, 2001; Kubota et al., 2006), *Reticulitermes hesperus* Banks (Haagsma & Rust 2005; Haverty et al., 2010), and *Reticulitermes flavipes* (Kollar) (Stansly et al., 2001; Su, 2014). However, the effectiveness of these baits when tested with higher termites was low (Neoh et al., 2011), particularly with the subfamily Macrotermitinae (Lee et al., 2014).

O. hainanensis Light belongs to the fungus-growing termites, the subfamily Macrotermitinae of the family Termitidae. Macrotermitinae has a complex food processing that establishes a symbiotic relationship with the fungus *Termitomyces*. Termites provide materials to build the fungus gardens that are a base for the growth of *Termitomyces* (Sieber, 1983; Nobre & Aanen, 2012), while the *Termitomyces* aid termites in the breakdown of cellulose and lignin into a more nutritious compost and supply the colonies with easily digestible, mineral-rich food, and at the same time play an important role in nest microclimate regulation (Sands 1969; Nguyen Van Quang, 2003; Hydro et al., 2003; Ahmad et al., 2021). The formation and development of any fungus garden is the food processing of the fungus-growing termites. The existence time of a fungus garden of

Macrotermes spp. can reach up to about 4 to 13 weeks (Nguyen Van Quang, 2003). The process of building the fungus gardens demonstrated a division of labor among termite castes, as well as among workers of different age groups (Badertscher et al., 1983; Hinze & Leuthold, 1999; Li et al., 2015).

Baits can be considered a food source for termites. Once termites consume the baits, toxic chemicals in the baits are transferred to other individuals in their colony, and consequently, the entire colony can be affected and eliminated. In order to define whether the use of bait is effective, it is necessary to thoroughly understand the processes of food searching, food receiving, food processing and food transferring in the colony of the targeted species. Data on these issues in species of the genus *Odontotermes*, particularly *O. hainanensis* is limited. Therefore, in order to establish a scientific basis to contribute to the study of developing more effective baits against this particular pest, our research focused on understanding the process of food processing and food distribution within *O. hainanensis* colonies.

MATERIALS AND METHODS

Research location

O. hainanensis termites were collected from nests in the section from Km 80–85 of the Red River dike in Hoang Mai - Thanh Tri, Ha Noi (20°55'03.2"N 105°53'13.6"E) and Linh Dam Park in Ha Noi (20°57'53.5"N 105°49'43.3"E), Vietnam. The separation, filtration, counting and setup of experiments were done in the laboratory of the Institute of Ecology and Works Protection. This study was conducted from July 2018 to September 2020.

Research Method

Food marking: food staining by mixing fluor-rot pigment particles (red pigment particles that are not dissolved in water and undigested in the termite intestine), with cellulose powder (d: 0.600 g/cm³, manufactured by Sigma-Aldrich (USA)) in a ratio of 0.5% (0.5g pigment/100 g cellulose

powder). This mixture is then added with water at 40% moisture and then used as termite food.

Distinguishing between castes and groups of workers: in a colony, termites usually have been divided into three main groups: reproductive termites (king, queen, and imagoes), labor termites (workers and soldiers) (Fig. 1a) and juvenile termites (white color and sizes varying by instars, Fig. 1b). As for the fungus - growing termites in general and the genus *Odontotermes* in particular, the workers are also classified into major workers (large in size) and minor workers (small in size). Based on the width of the head and the body color (Nguyen Van

Quang, 2003), the workers of *O. hainanensis* are classified into four specific groups as follows: young minor worker (small in size; 16-segment antennae, the 3rd segment is the shortest; no fat content on ventral and dorsal sides), old minor worker (small in size; 16-segment antennae, the 3rd segment is the shortest; high-fat content on ventral and dorsal sides), young major worker (large in size; 17-segment antennae, the 4th segment is the shortest; no fat content on ventral and dorsal sides) and old major worker (large in size; 17-segment antennae, the 4th segment is the shortest; high-fat content on ventral and dorsal sides) (Fig. 1a).

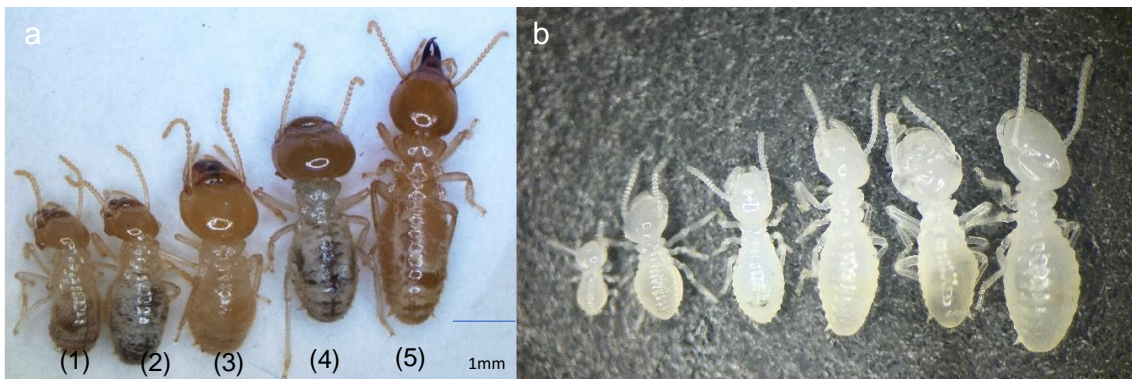


Figure 1. Some castes of *Odontotermes hainanensis*: a. (1): Young minor worker; (2): Old minor worker; (3): Young major worker; (4): Old major worker; (5): Soldier; b. Larvae [Photo by Nguyen Thi My]

Identifying the stages of fungus garden development: In *O. hainanensis*, old workers (including old major workers and old minor workers) participate in outside nest activities such as food foraging and carrying back to the nest, while young workers (including young major workers and young minor workers) accounted for a high proportion in the fungus garden cavities and was directly responsible for the building of the fungus gardens (Nguyen Thi My et al., 2021). In our experiment, termites and fungus gardens were collected from a field colony that was brought to the laboratory for separation into different groups of workers. Nine monitor units were

prepared with 100 young workers (50 young major workers, 50 young minor workers) for each in a plastic container (diameter: 50 mm, height: 30 mm) containing stained cellulose powder (40% moisture) and 0.5 g fungus garden. Daily monitoring of the termite activities in all units was conducted to record the time of the appearance of a new fungus garden (the red fungus gardens), the time of the appearance of fungus nodules (white fruiting bodies containing asexual spores of *Termitomices* spp.) on the red fungus garden, and the time when termites exploited the new fungus garden (there would gnaw marks on the red fungus garden).

In order to examine the development stages of the fungus garden between keeping under the laboratory and the field condition, we selected three nests of *O. hainanensis* based on the swarming hole areas in the dike. For each nest, a part of a fungus comb cavity was cut, then placed with 30g of stained cellulose powder in this cavity and covered with soil. After a period of 2, 7, 14 and 28 days, respectively, one fungus comb cavity was dig to investigate

and record the signs of the growth of the fungus garden built from the marked food (red).

The laboratory artificial rearing system: Based on Nguyen Van Quang (2003) and Li et al. (2015), the laboratory rearing model was created with plastic boxes (size 20 × 15 × 10 cm) with lids connected via transparent plastic tubes and connected to the termite activity observation area (arena) (Fig. 2).

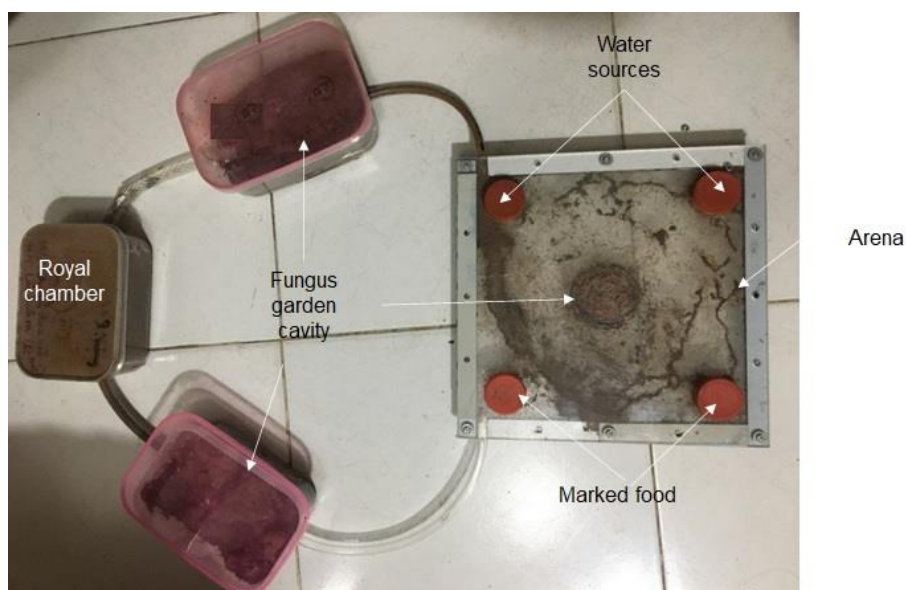


Figure 2. The laboratory artificial rearing system

Among the plastic boxes, one unit would act as the royal chamber (if the king and the queen were obtained), the rest would be compartments containing some lumps of the soil (taken from the wall of nests in the field), fungus gardens, and termites. The arena was a space made up of two square transparent plexiglass panels (sized 30 × 30cm) superimposed with a distance of 1cm, surrounded by plastic cushion bars 1cm thick, 2cm wide and fixed with screws. The area contained food boxes, moist soil and several small fungus combs. As the royal chamber and the fungus garden cavities were often covered with soil, it is necessary to create this area to facilitate the observation of termite activities.

Termites were collected from the field nest including workers, soldiers, larvae and reproductive termites (including the king, the queen and nymphs if possible) and the fungus gardens were brought back to be cultured in this model. The number of containers and the the number of fungus gardens in each container depended on the number of termite individuals obtained from the nest. This model was built to be able to observe with the naked eye, photograph and record termite activities such as feeding, sharing, spreading food and building fungus gardens, etc.

Experiments to determine the possibility of using the non-fungus garden feed: As only adult termites were involved in food foraging and processing, we examined whether baits

were capable of affecting the termite groups involved in feeding and building fungus gardens. Workers were divided into four groups (old major workers, young major workers, old minor workers and young minor workers) and 100 individuals from each group were kept in a plastic container (diameter: 50mm, height: 30mm) containing stained cellulose powder (40% moisture) and 0.5g fungus garden. After each period of 1h, 3h and 6h, 30 individuals in each group were dissected to check for the color pigment in their gut via stained food. The number of individuals with color pigment in different gut segments (foregut, midgut and hindgut) was determined. If the red color is found in two or three segments of the gut, this individual will be determined the stained food in the gut segment further from the mouth (for example, if color pigments are found in both the foregut and hindgut, the individual is determined the colored food in the hindgut). The experiment was repeated 3 times for each group of workers. The rate of color stain in each worker group was calculated.

Studying the ability to transfer food within the field colonies: based on the swarming hole areas, we determined the site of three *O. hainanensis* nests in the dike. For each nest, a part of a fungus comb cavity was cut and placed with 30 g of stained cellulose powder and covered with soil. After 1 week, the

dissections to examine the phenomenon of distributed marked food in the nests through the color of fungus gardens were performed. The percentage of fungus garden cavities that termites transfer food to is calculated using the following formula:

$$H(\%) = (A/B) \times 100$$

Where: H: Percentage of fungus combs with stained food; A: Number of fungus combs with stained food; B: Number of fungus combs obtained in a termite nest.

Data analysis: data are calculated using Microsoft Excel 2016.

RESULTS

The food processing of the *Odontotermes hainanensis* termites

The growth times of the fungus garden was presented in Table 1. It showed that the average time for the fungus garden to begin to be built was after 3.4 days of experiments (from the 2nd to the 6th day); the average time for the appearance of fungus nodules on the fungus garden was after 14.4 days of the experiment (from the 11th to the 18th day); and the average time for the fungus garden to begin to be utilized by termites was after 27.3 days (from the 21st to the 35th day). Thus, in the laboratory, the fungus gardens were grown from 21 days to 35 days, then reused by termites.

Table 1. The growing stages of fungus garden in laboratory rearing boxes

No.	Starting time (the days of experiment)			Growing stages of fungus garden (days)	
	A	B	C	B - A	C - B
1	4	14	21	10	7
2	2	14	25	12	11
3	6	18	29	12	11
4	2	11	28	9	17
5	3	13	31	10	18
6	2	12	23	10	11
7	4	18	35	14	17
8	5	17	29	12	12
9	3	13	25	10	12
Average	3.4 ± 1.1	14.4 ± 2.0	27.3 ± 3.3	11.4 ± 1.2	12.9 ± 2.8
Range	2-6	11-18	21-35	9-14	7-18

Notes: A: The new fungus garden starting to build up; B: The new fungus garden with nodules; C: The new fungus garden was re-eaten by the old workers.

In addition, monitoring results of termite feeding activities in the laboratory model (Fig. 2) showed that the old workers were responsible for food foraging, in which, the old major workers accounted for a larger proportion compared to the minor ones). These old workers left the nest to explore and search for the food source. When foods were located, old major workers cut and chew the pieces of food for a few minutes before carrying that food back to the nest. Initially, this task was involved by only a few individuals until the first piece of food

delivered to the nest, other old workers started locating to that food source to carry the food back (Fig. 3a). Food obtained by old workers from an outside source can be transferred to other old workers during delivering, or directly handed over young workers when backed to the nest (Fig. 3b), or gathered in piles under the fungus garden (Fig. 3c). The food was brought back to the nest then be used by young workers as materials to build up the fungus garden through their primary faecal pellets (Fig. 3d), that is the substance for growing of *Termitomyces* fungi.



Figure 3. Some activities of *Odontotermes hainanensis* workers in the food processing: a. The old workers were carrying food back to the nest; b. The forager transfers food for the young major worker; c. The food stored under the fungus garden before used to build up on the 2nd day of the experiment; d. The young major worker was depositing its faeces to build the new fungus garden [Photo by Nguyen Thi My]

Table 2. The growth of *Odontotermes hainanensis* fungus garden in the field nests

The days of experiment	Percentage (%) of nests with the following signs		
	Building a new fungus garden (the red fungus garden)	The new fungus garden with nodules	The new fungus garden with gnawing traces
2	66,7	-	-
7	100	-	-
14	100	100	-
21	100	100	-
28	100	100	33,3

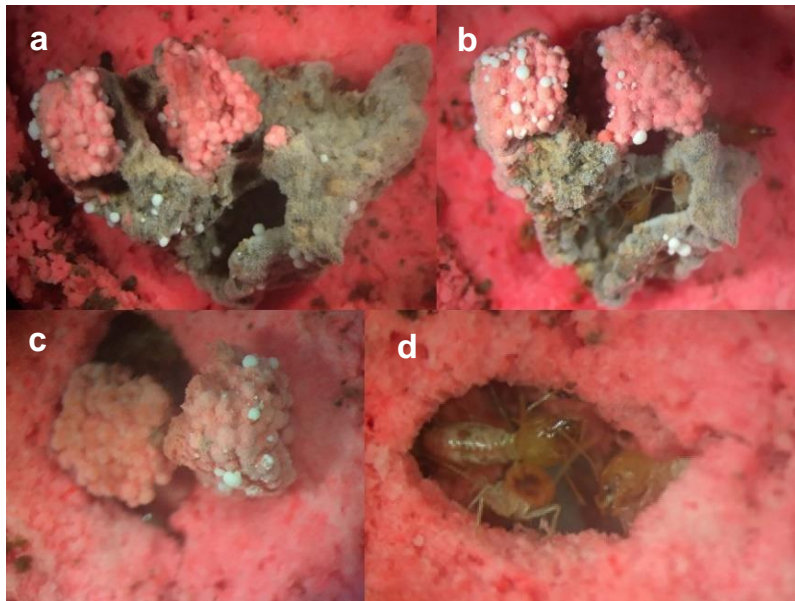


Figure 4. Growing stages of *Odontotermes hainanensis* fungus gardens in laboratory rearing boxes: a. New fungus garden (red) on the 7th day; b. The fungus garden with nodules on the 14th day; c. The old fungus garden was totally consumed after the 21st day; d. The fungus garden had been eaten up on the 28th day [Photo by Nguyen Thi My]

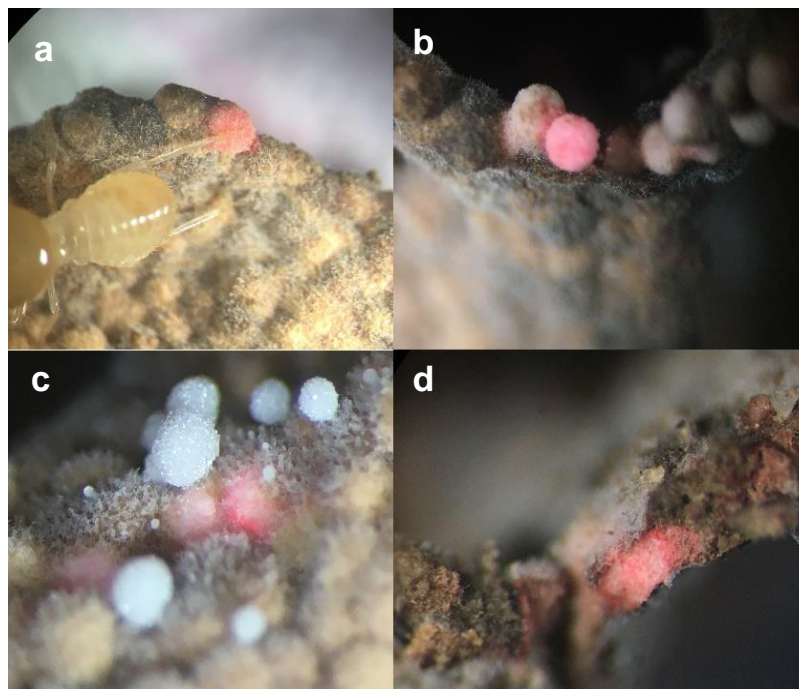


Figure 5. Growing stages of *Odontotermes hainanensis* fungus gardens in the field nests: a. The fungus garden on the 2nd day of the experiment; b. The fungus garden with a new one built up on the 7th day; c. The fungus garden with nodules on the 14th day; d. The new fungus garden with gnawing traces on the 28th day of the experiment [Photo by Nguyen Thi My]

The results on monitoring of the fungus garden growing in the field nests showed two nests with a cavity containing red fungus comb on the 2nd day, three nests with nodules on the red fungus comb on the 14th day and one nest with gnawing traces under the red fungus comb on 28th day of the experiment

(Table 2). The growth stages of the fungus gardens in the laboratory rearing boxes as well as in the field nests are more clearly in Figures 4, 5.

From the results of the above study, the mechanism of food processing of *O. hainanensis* can be summarized in Fig. 6.

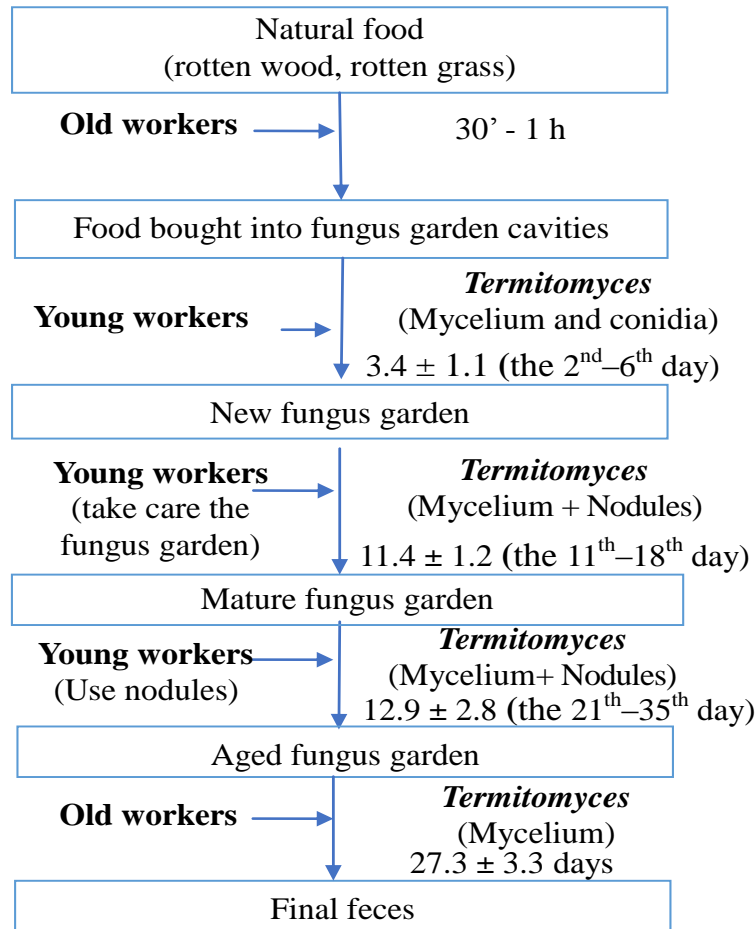


Figure 6. Diagram of food processing and consuming by *Odontotermes hainanensis* workers

The rate of termites with colored guts in each group of workers

This experiment was conducted to examine whether worker groups feed directly some foods collected in the field or bait. The results showed that all worker groups were able to directly use dyed cellulose powder in laboratory conditions. However, the level of marked food in their gut was different among worker groups

(Table 3, Fig. 7). After 1 hour, the percentage of workers with color stains in the guts was over 70% to 80% in each group, and increased after 3 hours of the experiment. After 6 hours of experiments, 100% of the young major worker group ate marked food and found colored guts while there remaining groups (old major workers, old minor workers and young minor workers) had a lower percentage of 93%, 80% and 90%, respectively (Table 3).

Table 3. Percentage of *Odontotermes hainanensis* workers with color stain gut in each group

Time of experiment	Gut sections	Percentage of workers with color contamination gut (%)			
		OMaW	YMaW	OMiW	YMiW
1 h	Foregut	60,0	43,3	56,7	66,7
	Midgut	10,0	16,7	23,3	13,3
	Hindgut	0,0	16,7	0,0	0,0
	Total	70,0	76,7	80,0	80,0
3 h	Foregut	16,7	6,7	6,7	30,0
	Midgut	36,7	13,3	43,3	23,3
	Hindgut	43,3	80,0	36,7	40,0
	Total	96,7	100,0	86,7	93,3
6 h	Foregut	0,0	0,0	0,0	3,3
	Midgut	0,0	0,0	6,7	10,0
	Hindgut	93,3	100,0	73,3	76,7
	Total	93,3	100,0	80,0	90,0

Notes: OMaW: Old major worker; YMaW: Young major worker; OMiW: Old minor worker; YMiW: Young minor worker).

Observations of dissected workers guts also showed that the amount of colored food in the worker groups was different. After 6 hours of experiments, the amount of marked food used by young major workers was the

highest proportion and almost these young major workers guts were full of marked food, while the amount of marked food found in the guts of old minor workers and young minor workers was minimal (Fig. 7).



Figure 7. The color stain in the guts of each worker group after 6h of the experiment: a. Gut of old major worker; b. Gut of young major worker; c. Gut of old minor worker; d. Gut of young minor worker [Photos by Nguyen Thi My]

Food transferring of *Odontotermes hainanensis* in field colonies

To examine the ability of food transferring in the field colonies, three nests of *O.*

hainanensis were selected. The results showed that marked food was distributed to almost the fungus comb cavities in all three nests with an average percentage of 95.78% (ranged from 92.11% to 100%) (Table 4).

Table 4. Percentage of fungus garden cavities detected stained food in the field of *Odontotermes hainanensis* colonies

Termite Nest	The number of fungus garden cavities	Fungus garden cavity found stained food	
		Number (cavity)	Percentage (%)
Nest 1	38	35	92.1
Nest 2	17	17	100
Nest 3	21	20	95.2
Average	25.3 ± 6.4	24.0 ± 5.6	95.8 ± 2.3

Moreover, the results also showed that the distribution of colored particles in fungus gardens was different between nests. The density of colored particles on the fungus

garden of nest 1, which had a high number of fungus gardens was sparser compared to nest 2 with the lower number of fungus combs (Fig. 8).

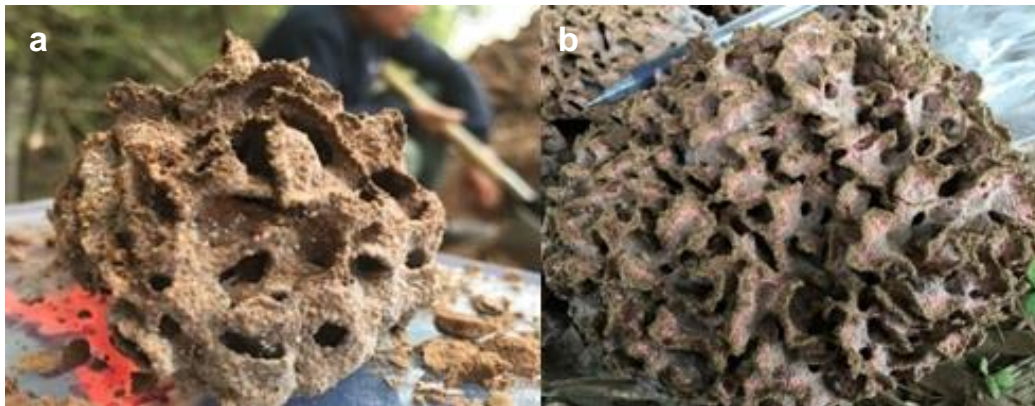


Figure 8. Distribution of red faecal pellets in *Odontotermes hainanensis* fungus garden after one week of the experiment (a. Nest 1; b. Nest 2) [Photos by Nguyen Thi My]

DISCUSSION

The results of the study on food processing demonstrated that the fungus garden of *O. hainanensis* went through 3 main stages: young fungus garden, mature fungus garden and aged fungus garden. Food processing had a division of labors between old and young workers. Old workers foraged and carried food to the nest, then young workers utilized it to build new fungus gardens based on primary faecal pellets. The new fungus garden was built on the aged fungus garden, thus the *Termitomyces* mycelium in the aged fungus garden would spread and grow on the new fungus garden. Moreover, *Termitomyces* also are transmitted via primary faeces of young workers as these termites ingest the vegetable matter collected by the foragers, and also feed on the nodules,

which contain asexual *Termitomyces* fungus spore during the first gut passage (Leuthold et al., 1989; Nobre et al., 2011). The young fungus garden stage was formed after about 2–6 days of experiments and takes place between 9 and 14 days, with an average of 11.4 ± 1.2 days. The mature fungus garden stage was counted from the appearance of fungus Nodules, which are white spherules growing in fungus gardens. Nodules contain asexual fungus spores (conidia) and are nutrient enriched food for young workers and larvae (Chiu et al., 2019). This stage started after 11–18 days of experiments and lasts for 7–18 days with an average of 12.9 ± 2.8 days. Following, the aged fungus garden stage was determined when the asexual reproduction fruits disappeared, and this fungus garden was used by old workers. Thus, the fungus garden

of *O. hainanensis* grew within 21 to 35 days (3 to 5 weeks) before the old workers reuse it.

The time to be reused by *O. hainanensis* was at least one week earlier than other *Macrotermes* studies. Josen (1971) studied 4 species of *Macrotermes* and concluded the average age of a fungus garden can reach up to about 5 to 7 weeks (as cited in Nguyen Van Quang, 2003). Nguyen Van Quang (2003) presented that workers of the *Macrotermes annandalei* species reused fungus gardens after they had been built for approximately between 4 and 13 weeks. This is probably due to the roles of symbiotic fungi being different between *Odontotermes* and *Macrotermes*. In *Macrotermes* spp., the main role of symbiotic fungi is to degrade lignin, so that the termites can utilize cellulose more efficiently, whereas, in *Odontotermes* spp., it is to serve as a food source (Hydro et al., 2003). According to Nguyen Van Quang (2003), in *M. annandalei*, food took 4 to 6 hours to pass through the middle gut and persisted weekly in the hindgut. Meanwhile, food through the guts of the *Odontotermes formosanus* was only about 3.5 h (Li et al., 2017; Chiu et al., 2019). These data showed that the process of digesting food through the termite guts is different in termite species.

The results of tests for the ability to use non-fungus garden feed through marked food showed that the guts of all groups of termites were found colored food. However, the amount of food and the level of color in each group of termites were different. The young major workers had the highest level of colored food, and food passing through their guts was faster than the rest worker groups. Particularly, during the first hour of the experiment, why colored food reached only the foregut and partial of the midgut in most groups of workers, it reached the hindgut of 16.7% of the young major worker group. After 3 hours of experiments, all worker groups had individuals containing colored food in the hindgut, however, the proportion of these individuals in the young major worker group was the highest, up to 80% of the total number of individuals. After 6 hours,

most individuals in worker groups whose hindguts contained colored food, however, the food was only in the anterior or middle of the hindguts, while most of the young major workers, stained food moved to the rectum. Moreover, based on color, it is possible to assess the degree of color stain in each worker group. After 6 hours of experiments, almost all of the guts of young major workers were red. While the guts of the old major workers and the old minor workers contained solid and dark-colored ingredients, the guts of young minor workers contained liquid food with transparent or opaque color. Thus, it can be seen that all groups of workers were capable of using and ingesting part of the food, which was carried back to the nest by the foragers, however, the level of this food in their guts varied among worker groups. This could imply that they are capable of being poisoned if they exploit the food source of poison baits.

The results of the study on food distribution in the field nests showed that the possibility of food transferring of *O. hainanensis* in the nest was very high, the proportion of fungal garden cavities with marked food in all test nests was more than 90%. Food placing in a cavity of the nest was distributed by termites to other fungus garden cavities to build a new fungus garden. The results also showed that the distribution of red color faecal pellets in fungus gardens was not the same among different nests. In particular, the density of colored pigment on fungus gardens in these nests consisting of a high number of fungus garden cavities was sparser than that of these low number of fungus garden cavities nests. This phenomenon could be explained either by the age of the termite nest, which affected the ability to utilize and transfer food within the colony, or the growing state of the fungus garden in each colony.

The above data showed that it is possible to apply poisonous baits to treat the colony of *O. hainanensis* through the distribution of food containing toxic active ingredients by adult workers. However, the colony *O. hainanensis* has a strict division of labor by

castes and age of workers. Only adult termites were involved in the process of foraging and food processing. In particular, old workers (old major workers and old minor workers) participate in external activities such as feeding, nest building repairing and cleaning, while young workers consisted of young major and young minor workers involved in activities such as building fungus gardens, tending reproductive termites and larvae. Because adult workers do not moulting, an active inhibitor of chitin synthesis does not have a direct effect on these termite groups. The active ingredients can only indirectly affect the remaining groups such as larvae, nymphs through worker groups and produce fungus gardens (nodules and old fungus gardens). Food carried by old workers from outside back to the nest was not continuously transferred among individuals in the colony because it had to go through a stage of utilisation to build a fungus garden. During this stage, the active ingredients can be degraded by the symbiotic fungi (Neoh et al., 2011; Chiu et al., 2021). Therefore, if baits (poisonous food) were used to control *O. hainanensis*, the spread of poisonous baits in the colony will be interrupted (the stage in the fungus garden). Nodules were consumed by the young worker and the fungus garden period was reused as food following at least 11 days and 21 days respectively in *O. hainanensis*. As a result, the number of individuals in the colony infected with baits will be small, leading to low treatment efficiency. This is the scientific basis to help us understand the fact that in many cases the use of chitin synthesis inhibitor baits has low efficiency, instability or takes a long time to handle the Macrotermitinae in general.

In short, research data on food processing, particularly the division of labor among adult worker groups and the ability of food spreading in *O. hainanensis*, serve as a basis to support the feasibility of further studying bait application in the treatment of *O. hainanensis* in particular and Macrotermitinae in general. However, further research is needed for this baiting technology to be

applied as well as the kind of active ingredient used for bait.

CONCLUSION

The research on food processing showed that only adult workers were involved in this task; all groups of workers consisted of old major workers, young major workers, old minor workers and young minor workers were capable of directly using the food that was cellulose powder introduced into the nest; the fungus garden was built by young workers based on their faecal pellets and grew between 21 to 35 days (3 to 5 weeks), then it was reused by old workers. The research on the distribution of food within field colonies of *O. hainanensis* species showed that marked food was transferred to the most of fungus garden cavities of the nest with more than 90% (from 92.1% to 100%) of the fungal garden cavities found marked food. These results are demonstrations of the feasibility of using baits to treat this species.

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