Chapter 4
The Diversity of Soil Fungus in and Around Termite Mounds of *Globitermes sulphureus* (Haviland) (Blattodea: Termitidae) and Response of Subterranean Termite to Fungi

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Abstract Many researchers are trying to develop fungus-based biological control methods against insect pests, including termites. This study explored the termite−fungus relationship using *Globitermes sulphureus* (Haviland) as a model species. Fungal species in termite mounds of *G. sulphureus* were isolated, purified, and identified. These fungal species were then introduced to termites, and their interactions were characterized. The preliminary study found 24 species of fungus from 10 locations in and around the *G. sulphureus* mound, with the most common being 5 species belonging to *Trichoderma* sp., *Aspergillus* spp., and *Penicillium* spp. We found that termites practice a symbiont relationship with the five species of soil fungi with which they were experimented.

4.1 Introduction

Termites are related to the cockroach family and are regarded as pests. Since termite food is mainly wood and woody tissue, this insect causes many problems for wood users. Most termite species feed on dead plant materials above, at, or

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under the soil surface and perform vital ecological roles in litter breakdown and in the recycling of mineral elements (Lavelle and Spain 2001), including dead foliage of grasses and other types of vegetation, woody materials such as roots and seeds, feces of higher animals, and other materials. As social insects, termites build nests to protect the entire colony from extreme climates and to defend against predators. In some species of termite, the nest functions as a place to store food (Noirot 1959; Thorne et al. 1996). Noirot and Johana (2000) identified three types of termite nest: subterranean (below ground level), epigeal (above the soil), and arboreal (on a trunk or tree branch and connected to the soil by crating mud tubes).

Globitermes sulphureus (Haviland) is commonly found in Malaysia, Singapore, and Vietnam. It has been classified under the family Termitidae. This species, which is highly prevalent in Malaysia, is easily identified based on the bright-yellow abdomens of the soldiers (Lee et al. 2003). It is a mound-building termite and builds epigeal nests. Noirot (1959) has categorized the mound of *G. sulphureus* as five layers: a royal chamber, pressed wood areas, the internal zone of the wall, the external layer of the wall, and the outer portion (bark). *G. Sulphureus* forage in the sand, feed on dead wood, and sometimes attack living trees and buildings (Roonwal 1970).

However, *G. sulphureus* is also beneficial to the environment as it breaks down wood debris and returns it to the soil. Besides termites, other microorganisms, such as fungi, also play important roles in decomposing dead wood and grasses. There are three functional groups of fungi: decomposers, mutualists, and pathogens. Around 100,000 out of 1.5 million estimated fungal species have been described by taxonomists (Hawksworth et al. 1995; Hawksworth 1991). Microbial populations in the soil are very diverse. Torsvik et al. (1996) calculated the presence of approximately 6000 bacterial genomes per gram of soil by taking the genome size of *Escherichia coli* (Migula) Castellani and Chalmers as a unit.

Being subterranean, *G. sulphureus* is exposed to various fungi and other pathogens that are abundant in the soil. Soil is a complex ecosystem where microorganisms live in heterogeneous communities, whereas the behaviors of individual species are often unknown due to the lack of suitable detection and identification techniques (Akkermans et al. 1994). Fungi play important physiological, mediative, and ecological roles in this ecosystem (Miller 1995). These roles include the decomposition of woody organic matter, the increase in nutrient uptake, the enhancement of plant resilience, and the improvement of soil structure (Jenkins 2005). In addition, some soil fungi have different functions and roles, and some are even harmful to other organisms, including termites.

Many studies have examined the interactions between termites and soil fungi (Sands 1970; Wong and Cheok 2001; Rosengaus et al. 2003). These relationships can be either symbiotic, attractant, antagonistic, pathogenic, or parasitic (Wong and Cheok 2001). More than 50 species of brown-rot fungi interact with subterranean termites, and these fungi can modify the nutrition, nest construction, survival, and caste development of termites (Amburgey and Beal 1977). The present research was carried out to investigate the number of fungal species and their populations from the inner, outer, and surrounding areas of mounds of *G. sulphureus* (Haviland)

and to focus on the interactions and relationships between subterranean *G. sulphureus* and soil fungi that were isolated from the termite nest.

4.2 Materials and Methods

4.2.1 Sampling of Nest Material

G. sulphureus nest materials were sampled from ten mounds at different locations on Penang Island, Malaysia. The height and width of the base of each mound were measured and recorded. Samples were taken from the inner and outer parts of the mound, and three samples (labeled A, B, and C) were taken from each section. The inner materials, taken from the second layer of the nest, 25–30 cm from the external wall, were dark brown and located near the royal chamber. The outer materials were taken from the fourth layer of the nest (internal wall), 5–7 cm from the external wall, and were dark and hard. Adjacent soil was sampled at 10–20 cm from each mound; three sections around the circumference of the mound were taken at depths of 5–15 cm. All the samples were kept in sterile paper bags and were then crushed with mortar porcelain until the maximum size of the material was 2 mm. All procedures were carried out under sterile conditions.

4.2.2 Isolation, Purification, and Identification of Fungi

Dilution plating was used to isolate fungi from the *G. sulphureus* carton. Five grams of *G. sulphureus* carton (inner and outer) and adjacent soil around the nest was mixed in 45 mL of sterile distilled water, shaken for 20 min, and kept under room conditions for 1 h to make a homogeneous suspension and to scrape the spores from the samples. Next, 10^{-2} and 10^{-3} dilutions were made by diluting 1 mL of suspension in 9 mL of sterile distilled water. Fifty microliters of suspension from each dilution was spread evenly over Rose Bengal Agar (RBA) surface with a sterile bent glass rod. The rod was sterilized by flaming with 95% ethanol before use. The inoculated dishes were incubated for 5 days at room temperature (25 ± 2 °C), after which the fungal colonies were counted. Each species of fungus was isolated and transferred into new PDA medium. Single-spore isolation was used to obtain pure fungal isolates. A piece of fungus was transferred to and diluted in sterile water and shaken. Fifty microliters of suspension was transferred on WA medium and incubated for 3 days, after which the single pure colony was removed and transferred on PDA medium to a petri dish. Morphological identification was made according to Moore-Landecker (1996). Microscopic observations were made by measuring the size of the hyphae and spores of the fungi under a compound microscope. The morphological identification was done up to the genus level.

Table 4.1 Observation of termite activity and fungal growth scores

Score	Termites	Fungi
1	90–100% dead	Fungi have not grown
2	Moribund, near death, still moving their antennae	Fungi growing, covering 10–20% of the bottom of the substrate
3	Not active, still moving slowly, grooming each other and themselves; 50–70% of termites dead	Fungi growing, covering 20–50% of the bottom of the substrate
4	Active, moving around the nest, grooming each other and themselves, collecting food; 20–50% of termites dead	Fungi growing, covering all of the bottom of the substrate and half of the surface area; 50–80% of the substrate overall
5	Active (healthy), moving around the nest, grooming each other and themselves, collecting food; <20% of termites dead	Fungi growing, covering 80–100% of the substrate overall, including all of the bottom and almost all of the surface

4.2.3 Response of G. sulphureus *to Fungi*

The impact of fungal concentration was a factor in this test. The experiment was conducted inside a glass jar (80 mm diameter x 150 mm height), termite carton (<20 mesh) as medium, and 40% water content. Five common fungal species isolated from the nest material were used in this test. These species were cultured in the lab on PDA medium. Two-hundred workers and 20 soldiers were used for each test. The activities of the termites and fungi were observed every 2 days up to 14 days. The observations of termite activity and fungal growth were done by giving a score for each level of termite activity and of fungal germination, respectively. The score ranges were 1–5 (see Table 4.1).

4.2.4 Statistical Analysis

All analyses were performed using SPSS version 16.0 software. Differences were tested by analysis of variance (ANOVA) and subsequent post hoc comparisons (Tukey HSD test). All tests were performed at a 95% confidence interval.

4.3 Results and Discussion

Detecting exactly which fungi were present in a soil sample was not easy, primarily because of the fastidious nature of the great majority of termite species. Hawksworth (1991) reported that there are 1.5 million species of fungi in soil and that only 10% of them have been described. Soil fungi are a group of microorganisms that can actively grow in close association with other organisms or can exist in a dormant form. Saprobic fungi are the largest group of fungal species that occur in

Table 4.2 Colony and species numbers from direct and dilution plating methods from *G. sulphureus* (Haviland) mound carton and adjacent soil

Sampled part of the mound	Dilution plating method		Direct plating method	
	Species number	CFU/gram soil (×1000)	Species number	CFU/gram soil (×1000)
Inner part	4.20 ± 0.92	46.90 ± 26.66	3.00 ± 0.95	–
Outer part	6.60 ± 1.07	31.51 ± 2.32	4.30 ± 0.95	–
Adjacent soil	9.50 ± 1.08	9.29 ± 1.55	4.29 ± 1.03	–

soil, and they have an important role in the decomposition of cellulose, hemicellulose, and lignin compounds, which in turn contribute to the carbon cycle (Mahmood et al. 2006; Saravanakumar and Kaviyarasan 2000). In addition, these catabolic activities enable fungi to grow in innutritious substrates. A lot of studies, using various methods, have described the diversity of soil fungi (Viaud et al. 2000; Bridge and Spooner 2001; Hawksworth 2001; Celine et al. 2004). Yet, few studies have described the diversity of soil fungi and the interaction between fungi and termites. This report discusses the diversity of the fungal community in *G. sulphureus* mounds as determined by isolating, culturing, and counting colonies using the dilution plating method.

Fungal species composition from the termite carton and adjacent soil can be analyzed by either the direct or the dilution plating method. Our preliminary study showed that the number of fungal species from the dilution plating method was more diverse compared to the direct plating method (Table 4.2). The fungal species grown by direct plating were also found by the dilution plating method, so the latter was used to isolate fungal species in *G. sulphureus* mounds.

Dilution plating is a method to isolate a fungus by immersing a sample in sterile water and culturing the fungus. Here, dilution plating showed that the inner part of a mound contains fewer species with high colony numbers. As for the outer part, this method revealed high numbers of fungal species but with low colony numbers. The different numbers of fungal species from the inner and outer parts could be related to the moisture content of the soil. It is supported by the data of *G. sulphureus* nest, which are the moisture content of the *G. sulphureus* nest at the inner part of the mound was higher than that at the outer part or in the adjacent soil24.

Based on the results shown in Table 4.2, the isolation of fungal species from *G. sulphureus* mounds was carried out using the dilution plating method, and RRBA medium was used to grow the fungi. Figure 4.1 shows the numbers of colonies and species of soil fungi from each sampling spot (inner, outer, and adjacent). The inner part of the mound was less diverse than the outer or the adjacent soil. In previous studies, the species number suggested that the inner, outer, and adjacent samples had least to most diversity, in that order. The mean species number from the inner part was less than that of the outer part or that of the adjacent soil: 3.55 ± 0.21, 5.82 ± 0.38, and 7.08 ± 0.45 species, respectively. On the other hand, the inner part had more colonies than either the outer or adjacent part: 4.77 ± 0.56, 2.44 ± 0.14, and 1.19 ± 0.31, respectively.

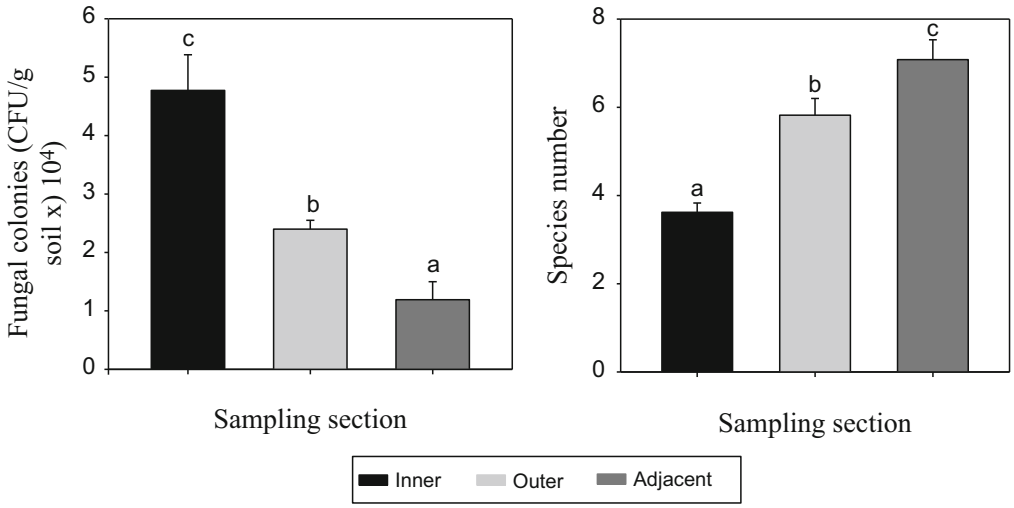

Fig. 4.1 Fungal colony and species numbers from three-sample section (inner, outer, and adjacent soil) of *G. sulphureus* mound

The growth of fungal species was correlated with the nutrient content in *G. sulphureus* mounds, each part of which had a different nutrient composition. The inner part contained more cellulose and nitrogen compared to the outer part (Guswenrivo et al. 2011). Griffin (1972) reported that carbon, nitrogen, and minor nutrients (such as phosphorus and sulfur) are crucial for fungal growth, as is the ratio between carbon and nitrogen. Fungal growth is known to be affected by other factors, as well, such as moisture, temperature, the quality of resources, competition, and local processes such as predation and other biota (Celine et al. 2004). The *G. sulphureus* nest is built from soil together with their saliva and also from undigested food that is rich in nutrients such as nitrogen and carbon (Krishna and Weesner 1970; Brauman 2000). In other words, the inner part of the mound possesses optimal conditions (humidity and acidity) for fungal growth.

All of those conditions should support fungal growth, but the present results turn out to be different. The inner part of the mound, which is highly suitable for fungal growth, lacked fungal populations when compared to the outer part or even the adjacent soil. This was because the termites concentrated in the center of the nest. Other factors have also been found to affect and inhibit fungal growth.

The number of termites present in a nest affects fungal availability, since *G. sulphureus* grooms in the nest. Grooming is a social behavior; termites clean their bodies, the nest, and their nestmates. Termite saliva reportedly contains an antifungal component that can inhibit fungal growth. In light of this, some fungal species were not expected to survive or might exist in a dormant stage. Another factor is the competition for food sources between *G. sulphureus* and fungi. The center part of the nest was full of termites (workers, soldiers, and nymphs), so fungal growth in the mound would be restricted. Overall, 11 species of fungi were isolated in the inner part, 15 species in the outer part, and 24 in the adjacent soil.

Table 4.3 Diversity of fungal species isolated from inner part, outer part, and adjacent soil of *G. sulphureus* (Haviland)

No.	Sample area	Group	Genus
1.	Inner part	*Ascomycota*	*Aspergillus* sp. (two species)
			Penicillium sp. (four species)
			Trichoderma sp. (one species)
		Zygomycota	*Mucor* sp. (one species)
		Unidentified fungi (three species)	
2.	Outer part	Ascomycota	*Aspergillus* sp. (two species)
			Penicillium sp. (six species)
			Trichoderma sp. (one species)
			Fusarium sp. (one species)
		Zygomycota	*Mucor* sp. (one species)
		Unidentified fungi (four species)	
3.	Adjacent soil	*Ascomycetes*	*Aspergillus* sp. (two species)
			Penicillium sp. (nine species)
			Trichoderma sp. (one species)
			Fusarium sp. (one species)
		Zygomycota	*Mucor* sp. (one species)
		Unidentified fungi (ten species)	

The five common fungal species isolated from the nest material and adjacent soil included one species of *Trichoderma*, two of *Aspergillus*, and two of *Penicillium*.

Table 4.3 shows that most fungal species in the inner part were from groups of Ascomycetes. Fungi such as *Trichoderma* sp., *Aspergillus* sp., and *Penicillium* sp. were the dominant species of fungi isolated from all three locations. Being subterranean, *G. sulphureus* colonies are exposed to various fungi and other pathogens that can be found in the soil. In interactions between fungi and termites, fungi might function as symbionts, attractants, antagonists, pathogens, or even parasites to the termites (Wong and Cheok 2001). This was confirmed by Corinne (2000), who stated that the presence of fungi in a termite nest can include compounds that attract or repel termites and can also affect termite nutrition. *Gloeophyllum trabeum* and *Serpula lacrymans* each contain a substance attractive to termites (Esenther et al. 1961; Matsumura et al. 1969; Sugamoto et al. 1990). Smythe et al. (1970) reported that *Reticulitermes flavipes* survived better on decayed wood than on sound wood. This was due to some fungal species having modified fatty acids, affecting their protein and amino acid compositions or even serving as a food source for *R. flavipes* (Carter et al. 1972; Carter and Smythe 1973; Waller et al. 1987; Waller 1993).

Subterranean termites live in soil, together with more than one million species of fungi. Termites live in colonies with high-density populations and humid conditions, making them susceptible to fungal infection (Vargo et al. 2003; Yanagawa and Shimizu 2007). However, there is a lack of information on interactions between termites and soil fungi. Most information concerns interactions and relationships

Table 4.4 Termite mortality and food consumption (%) during exposure to soil fungi at four different incubation times after inoculation (in weeks)

Observation factor	Age	Fungal species					
		Trichoderma sp.	*Aspergillus* sp. (B)	*Aspergillus* sp. (G)	*Penicillium* sp. (A)	*Penicillium* sp. (B)	Control
Termite mortality by week (%)[1]	0	50.4 ± 42.47 a	48.5 ± 14.22 a	68.4 ± 11.12 a	78.9 ± 14.15 a	80.89 ± 13.89 a	26.5 ± 14.88
	1	100.0± 0 b	100.0± 0 b	100.0± 0 b	100.0± 0 b	100.0± 0 b	
	2	100.0± 0 b	100.0± 0 b	100.0± 0 b	55.7 ± 12.52 b	77.9 ± 17.55 b	
	4	100.0± 0 b	100.0± 0 b	100.0± 0 b	100.0± 0 b	100.0± 0 b	
Food consumption by week (%)[1]	0	2.42 ± 0.60 b	2.38 ± 0.43 b	2.47 ± 0.27 b	2.28 ± 0.32 c	2.21 ± 0.43 c	6.20 ± 0.76
	1	0.63 ± 0.22 a	0.54 ± 0.18 a	0.75 ± 0.20 a	0.76 ± 0.12 a	0.71 ± 0.22 a	
	2	0.68 ± 0.20 a	0.77 ± 0.20 a	0.66 ± 0.35 a	1.18 ± 0.35 b	1.24 ± 0.47 b	
	4	0.70 ± 0.11 a	0.57 ± 0.16 a	0.57 ± 0.57 a	0.48 ± 0.06 a	0.65 ± 0.65 a	

[1]Means followed by the same letter within the same row are not significantly different ($P > 0.05$; Tukey HSD), $N = 5$

between Macrotermitinae and a fungus-growing termites subfamily of Termitinae (Aanen et al. 2007), and other Termitinae subfamilies, and also on the symbiosis between termites and wood decay (Cornelius et al. 2002). An experiment on the interaction between *G. sulphureus* and soil fungi was conducted for a period of 14 days. Termite activities such as grooming behavior, movement, mortality, and food consumption were recorded. Table 4.4 shows the mortality and food consumption activities of *G. sulphureus* after the termites were exposed to five species of soil fungi of four different fungal growth ages after the fungi were inoculated after that period.

Apparently, the age of a soil fungus affects termite mortality. Their ability to survive and their resilience on substrates containing fungi were tested with five different species of soil fungi in four different age groups (0, 1, 2, and 4 weeks). Termites practice hygiene and grooming activities to protect themselves and the colony from contamination. Beginning on the first day of a fungal incubation period, the fungus grew very productively, with both an increasing volume of individual cells, increasing overall numbers of cells, and growing biomass (Reeslev and Kjøller 1995). During the second week of incubation, fungi would start to secrete secondary metabolites (enzymes) that may be useful, harmful, or toxic to other organisms (Maheshwari et al. 2000). Each fungus secreted enzymes at different rates, e.g., *Phanerochaete* starts to increase its enzyme secretion after 8 days of incubation (Johnson et al. 1994), and *Colletotrichum gloeosporioides* started to secrete enzymes after 4 days of incubation (Onofre et al. 2011). Fungi began to decrease their production of secondary metabolites when they reached 4 weeks of incubation.

Termite mortality on growth day 0 of each fungal species differed significantly ($P < 0.05$) depending on the fungal incubation period, i.e., after 1, 2, and 4 weeks. This was supported by the data on the termites' consumption of a wood block, which differed significantly ($P < 0.05$) among the 1-, 2-, and 4-week fungal growth periods; at fungal growth day 0, *G. sulphureus* consumed more wood than at the other time points (Table 4.4). The observation of termite interactions showed that the survival rate of *G. sulphureus* decreased with increasing time. Termite mortality increased over time, reaching 100% at the 14-day observation after being exposed to fungi at 1, 2, and 4 weeks of growth, whereas for termites exposed to fungi on growth day 0, mortality of termite at the 14-day observation ranged between 48% and 80%. These differences in termite mortality depending on the length of time after incubation were caused by the compounds released by the fungi that influenced the interactions between them and the termites. These compounds are commonly associated with the sporulation process in microorganisms, including fungi, and are often called secondary metabolites (Bu'Lock 1961; Sekiguchi and Gaucher 1977). Fungi usually begin producing these compounds upon entering a stationary or resting phase (Bu'Lock 1961).

On the first day of observation, there were high numbers of healthy termites, but the number gradually decreased up to day 14. This trend was demonstrated by the activities of the termites. The activity levels and healthiness of the termites decreased with time (Fig. 4.2), as reflected in the decreasing numbers of healthy

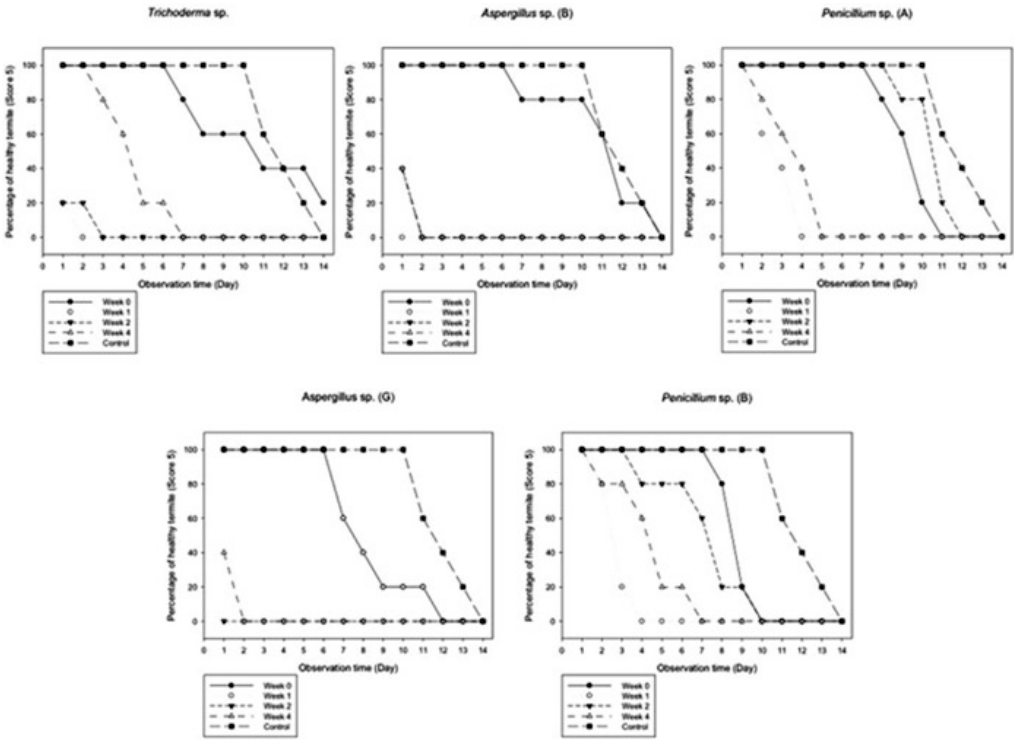

Fig. 4.2 The activity of *G. sulphureus* Haviland upon exposure to five different species of fungi (*Trichoderma* sp., *Aspergillus* sp. (A), *Aspergillus* sp. (B), *Penicillium* sp. (A), *Penicillium* sp. (B)) at different incubation periods after inoculation based on the percentage of healthy termites (score 5)

termites that still survived. The movement of termites and their grooming activities began to decline. Fungal growth increased with time, as indicated by the presence of mycelia and hyphae. Figure 4.2 shows the stage of fungal growth when the fungi came into contact with *G. sulphureus*. Due to the different lengths of time after incubation, there were different stages of fungal growth when the experiment was started. The 1-, 2-, and 4-week fungi completely covered the surface of the substrate for.

Among the factors that would influence the interactions between termites and soil fungi is the population density of fungi in the soil. In this chapter, two different population densities were tested, and termite mortality and food consumption were observed (Table 4.5). There was a significant difference ($P < 0.05$) between the two concentrations; 10^5 CFU of soil fungi was a concentration that *G. sulphureus* could tolerate, with the mortality rate varying according to the fungal species. However, at 10^7 CFU of soil fungi, termite mortality was 100% regardless of the fungal species (Table 4.5). On the other hand, food consumption did not differ significantly ($P > 0.05$) between the two concentrations (10^5 CFU and 10^7 CFU; Table 4.5).

The inner part of the *G. sulphureus* (Haviland) mound contains lower concentrations of soil fungi compared to the outer part and adjacent soil. This corroborated well a report by Chouvenc and Su (2010), where subterranean termites exhibited an

Table 4.5 Termite mortality (%) and food consumption after exposure to two concentrations (CFU) of soil fungi for 14 days

No.	Fungal species	N	Termite mortality (%)		Food consumption (%)	
			10^5 CFU	10^7 CFU	10^5 CFU	10^7 CFU
1	*Trichoderma* sp.	5	48.7 ± 35.28 a[1]	100.0± 0 b	2.61 ± 0.74 a[1]	1.20 ± 0.31 a
2	*Aspergillus* sp. (G)	5	94.5 ± 10.65 a	100.0± 0 b	1.50 ± 0.46 a	0.70 ± 0.05 a
3	*Aspergillus* sp. (B)	5	98.5 ± 2.12 a	100.0± 0 b	1.72 ± 0.23 a	0.64 ± 0.12 a
4	*Penicillium* sp. (A)	5	73.5 ± 11.07 a	100.0± 0 b	2.57 ± 0.32 a	0.79 ± 0.23 a
5	*Penicillium* sp. (B)	5	95.9 ± 3.66 a	100.0± 0 b	2.08 ± 0.39 a	0.78 ± 0.74 a
6	Control	5	27.5 ± 14.45		5.84 ± 0.81	

[1]Means followed by the same letter within the same row are not significantly different ($P > 0.05$; Tukey HSD)

ability to protect and defend themselves and their entire colony from fungi by three major defense mechanisms: grooming, cellular encapsulation, and gut antifungal activity. Those authors explained that the relationship between termites and entomopathogenic fungi is multilevel and that each level is a barrier against fungi that prevents fungi from thriving and completing their life cycle.

In another study, exposure to soil fungi elicited various behaviors from termites, including hygienic, attacking, cannibalistic, and burying behaviors (Noirot and Darlington Johana 2000). This observation showed that *G. sulphureus* workers removed fungal mycelia from the surrounding food sources. On the other hand, the observation also showed the termites groomed themselves as well as their nestmates. Yanagawa et al. (2009, 2010) found that the grooming activity of subterranean termite *C. formosanus* did not change when it came into contact with entomopathogenic fungi. Recently, Yanagawa (2011) reported that termite grooming activity differed significantly and increased when uninfected termites mixed with termites infected with entomopathogenic fungi. The termites exhibited this behavior from the very first day after being exposed to the infected termites, and termite was focused in areas where food was available. Termite mortality eventually increased with time. This also indicates that the termite survival was stronger at substrates contaminated with soil fungi.

Fungal growth can be inhibited by termites through hygienic and grooming activities (Fig. 4.3). This is because when termites groom, they use their saliva to cleanse themselves and their nestmates from fungal spores/mycelia that were attached to them. Lamberty et al. (2001) found that the saliva secreted by the higher termite *Pseudocanthotermes spiniger* during grooming contains the antifungal peptide termicin. The same was true of *Nasutitermes* sp., in whose saliva Bulmer and Crozier (2004) found a related antifungal peptide. This corroborated well the observation in this study that *G. sulphureus* inhibited the growth of soil fungi (fungi age 0 week) and cleaned the surface of the substrate from mycelia (fungal ages 1, 2, and 4 weeks). Fungi at age 0 week grew vines on the bottom of the substrate, while the substrate surface was not yet covered with fungal mycelia. With fungi at ages 1, 2, and 4 weeks, the substrate was fully covered with mycelia, but termites started

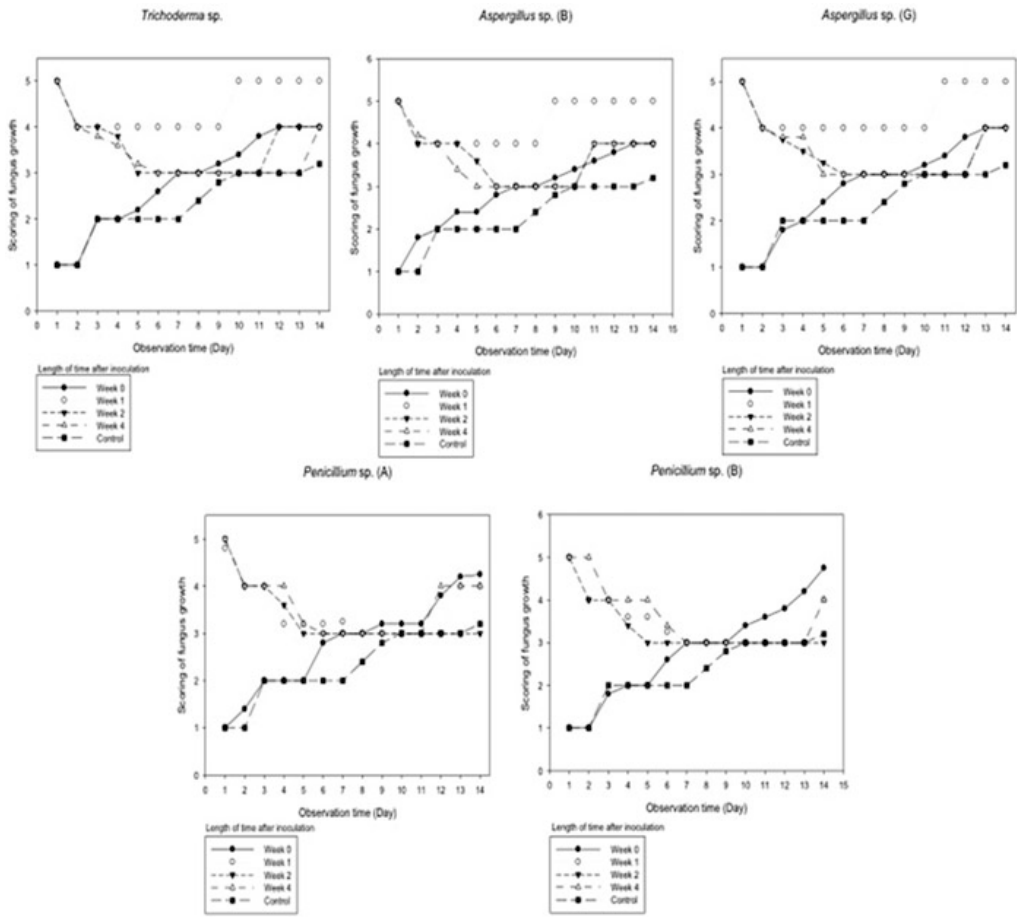

Fig. 4.3 Growth rates of fungi (*Trichoderma* sp., *Aspergillus* sp. (G), *Aspergillus* sp. (B), *Penicillium* sp. (A), and *Penicillium* sp. (B)) in the presence of *G. Sulphureus*

to clean up the substrate surface and moved the mycelia aside. After several days of exposure to fungi, *G. sulphureus* started to make tunnels under and around food sources. This indicated that *G. sulphureus* can protect themselves from contamination.

The antifungal activity was observed in this colony when *G. sulphureus* removed mycelia of soil fungi aside by picking them up with their mandibles and pushing them away from food sources. Some fungal spores will attach to the termite body, while others will enter the abdomen, which can kill a termite. Therefore, grooming activity, where nestmates clean each other with their glossae and excrete the spores after digesting them, plays an important role (Yanagawa and Shimizu 2005, 2007). The inhibition of fungal growth shown by the termite gut and/or termite body indicated that *G. sulphureus* produced and secreted antifungal compounds. Previous reports showed that the termite intestine has a fungistatic ability against hyphal growth and that the termite gut resists the growth of conidia once they pass through it (Bao and Yendol 1971; Kramm and West 1982; Boucias et al. 1996; Rosengaus et al. 1998; Siderhurst et al. 2005).

On the other hand, the concentration of fungal spores in soil also affects the activities and mortality of *G. sulphureus*. Previous studies showed that the presence of fungal spores at a particular concentration could increase the rate of termite mortality up to 100% within a few hours, whenever contact was possible between the termites and fungi (Yanagawa and Shimizu 2007). Yanagawa and Shimizu (2007) explained that a lower concentration of fungal conidia in substrate did not affect termite activity due to the termites' hygienic, grooming, and antifungal activities. *G. sulphureus* showed the same reactions when it was exposed to the lower of two concentrations of soil fungi (10^5 CFU) used in this study. Termite mortality was lower at 10^5 CFU than at 10^7 CFU. This shows that *G. sulphureus* was able to survive at this concentration of fungal spores through hygiene and grooming activity.

In fact, the inner part of the *G. sulphureus* mound has fewer species of soil fungi than the outer part of the mound or the adjacent soil. The reverse was true for the number of colonies: the inner part had more than the other parts. The Ascomycetes group, whose members include *Trichoderma* sp., *Aspergillus* sp., and *Penicillium* sp., among others, accounts for the majority of fungal species inside the mound. The different numbers of fungal species between the inner part, outer part, and adjacent soil of the *G. sulphureus* (Haviland) mound showed that the termites interact with soil fungi. The interactions of *G. sulphureus* with soil fungi have been tested with five species of soil fungi (*Trichoderma* sp., *Aspergillus* sp. (two species), and *Penicillium* sp. (two species)), revealing that the density of the fungi affected the termites' ability to stand up to and adapt to those fungi. In addition, the ability of *G. sulphureus* to survive and interact with soil fungi could be supported by the antifungal compounds produced in the termite gut.

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