EVALUATION OF TOXICITY, REPELLENCY EFFECTS AND PHYTOCHEMICAL OF SOME PLANT EXTRACTS AGAINST SUBTERRANEAN TERMITES, Coptotermes gestroi AND Globitermes sulphureus (BLATTODEA: RHINOTERMITIDAE; TERMITIDAE)

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by

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LIST OF ABBREVIATIONS

ºC	degree Celcius
%	percentage
/	per
cm	centimeter
g	gram
ml	milliliter
mm	millimeter
ppm	parts per million
RH	relative humidity
LT_{50}	lethal time required to kill 50% of population exposed
LT_{95}	lethal time required to kill 95% of population exposed
±	central range
X^2	chi square
>	more than
<	less than
RT	retention time

LIST OF APPENDICES

PENILAIAN KETOKSIKAN, KESAN MENGUSIR DAN FITOKIMIA DARIPADA BEBERAPA TUMBUH-TUMBUHAN EKSTRAK KE ATAS ANAI-ANAI BAWAH TANAH, *Coptotermes gestroi* DAN *Globitermes sulphureus* (BLATTODEA: RHINOTERMITIDAE; TERMITIDAE)

ABSTRAK

Anai-anai bawah tanah, *Coptotermes gestroi* (Wasmann) dan *Globitermes sulphureus* (Haviland) dikenali sebagai anai-anai perosak yang paling penting. Saringan bagi produk semula jadi yang mempunyai aktiviti anti-termitik mampu dijadikan sebagai alternatif yang berkesan dan sebagai racun serangga botani mesra alam. Ketoksikan dan aktiviti mengusir oleh ekstrak mentah dari pokok *Leucaena leucocephala* (Lam.) de Wit, *Andrographis paniculata* (Burm. f.) Wall. ex Nees, *Azadirachta indica* A. Juss dan *Phyllanthus niruri* Linnaeus yang diektrak dengan metanol, heksana dan air telah dikaji ke atas *C. gestroi* dan *G. sulphureus*. Ujian makmal, iaitu bioasai tiada pilihan dan bioasai pilihan telah dijalankan di dalam makmal dengan menguji semua ekstrak mentah dengan kepekatan yang berbeza, 500, 5,000 dan 10,000ppm. Selepas 14 hari, LT_{50} paling rendah oleh *G. sulpureus* dan *C. gestroi* ditemui dalam ekstrak metanol *P. niruri*, *L. leucocephala*, *A. paniculata* dan *A. indica* dengan kepekatan 10,000 ppm (*G. sulphureus*, LT_{50} = 147.696, 189.337, 270.863 dan 172.187 jam), (*C. gestroi*, LT_{50} = 89,450, 134.596, 148.534 dan 165.098 jam). Pilihan bioasai juga menunjukkan ekstrak metanol *P. niruri* pada kepekatan 10,000 ppm dapat mengusir anai-anai *C. gestroi* (80%) dan *G. sulphureus* (97.5%). Tiada pilihan bioasai telah dijalankan untuk mengkaji potensi kompaun semula jadi sebagai bahan racun serangga baru dengan menilai ketoksikan secara langsung kedua-

dua flavonoid (rutin dan quersetin) terhadap *C. gestroi* dan *G. sulphureus*. Hanya rutin menyebabkan kematian anai-anai yang tinggi dengan pengambilan makanan yang rendah. Aktiviti menerowong oleh *C. gestroi* dan *G. sulphureus* telah dijalankan melalui kaedah piring petri bagi menilai prestasi ekstrak mentah. Hasil kajian menunjukkan bahawa semua ekstrak mentah menunjukkan ciri-ciri bukan pengusir kecuali ekstrak pokok daripada metanol. Aplikasi GC-MS dan ujian fitokimia digunakan sebagai teknik untuk mengenalpasti konstituen meruap dalam ekstrak tumbuhan metanol. Ujian fitokimia mendedahkan bahawa *P. niruri*, *A. paniculata* dan *A. indica* mengandungi alkaloid, flavonoid, saponin, tanin dan triterpens/steroid, manakala kandungan ekstrak metanol daripada *L. leucocephala* terhad kepada alkaloid, flavonoid, saponin, dan triterpens/steroid. Daripada analisis GC-MS, kompoun meruap yang paling tinggi dijumpai daripada *A. indica*, *A*, *paniculata*, *L. leucocephala* dan *P. niruri* adalah phytol (48.26%), 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-(19.623%), alpha.-tocophorol-.beta.-D-mannoside (37.556%) dan dl-.alpha.-tocopherol (47.792%).

EVALUATION OF TOXICITY, REPELLENCY EFFECTS AND PHYTOCHEMICAL OF SOME PLANT EXTRACTS AGAINST SUBTERRANEAN TERMITES, *Coptotermes gestroi* AND *Globitermes sulphureus* (BLATTODEA: RHINOTERMITIDAE; TERMITIDAE)

ABSTRACT

The subterranean termites, *Coptotermes gestroi* (Wasmann) and *Globitermes sulphureus* (Haviland) are known as the most important termite pests. Screening for anti-termitic activity of natural products could be a promising alternative as an effective, environmental friendly botanical insecticides. Toxicity and repellency activity of crude methanol, hexane and water extracts of *Leucaena leucocephala* (Lam.) de Wit, *Andrographis paniculata* (Burm. f.) Wall. ex Nees, *Azadirachta indica* A. Juss and *Phyllanthus niruri* Linnaeus were investigated on *C. gestroi* and *G. sulphureus*. Laboratory bioassays, no choice and choice bioassay were conducted by testing all the crude extracts with different concentrations, 500, 5,000 and 10,000ppm in the laboratory conditions. The lowest LT_{50} of *G. sulpureus* ad *C. gestroi* were found in all methanol extract of 10,000 ppm of *P. niruri, L. leucocephala, A. paniculata* and *A. indica* after 14 days (*G. sulphureus*, LT_{50} = 147.696, 189.337, 270.863 and 172.187 hours), (*C. gestroi*, LT_{50} = 89,450, 134.596, 148.534 and 165.098 hours). Repellent activity in choice bioassay also showed that 10,000ppm of methanol extract of *P. niruri* caused 80% and 97.5% repellency for *C. gestroi* and *G. sulphureus* respectively. No choice bioassay was conducted to investigate the potential of natural compound as a new insecticide ingredient by assessing the direct toxicity of the two flavonoids (rutin and quercetin) against *C.*

gestroi and *G. sulphureus.* The result shows only rutin resulted with a high termite mortality with low feeding consumption compared to quercetin. Tunneling activity of *C. gestroi* and *G. sulphureus* was constructed through petri dish method to evaluate the performance of crude extracts. Results showed that with the exception of methanol plant extracts, all crude extracts demonstrated non-repellent properties. Application of GC-MS and phytochemical test used as a technique to identify the volatile constituents in methanol plant extracts. The phytochemical test revealed that *P. niruri*, *A. paniculata* and *A. indica* contained alkaloids, flavonoids, saponins, tannins and triterpens/steroid, while the methanol extract of *L. leucocephala* only limited to have alkaloids, flavonoids, saponins, and triterpens/steroid. From the GC-MS analysis, the most prevailing volatile compound from *A. indica, A, paniculata, L. leucocephala* and *P. niruri* was phytol (48.26%), 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-(19.623%), alpha.-tocophorol-.beta.-D-mannoside (37.556%) and dl-.alpha.-tocopherol (47.792%) respectively.

CHAPTER 1

INTRODUCTION

Among the social insects, subterranean termites are widely known as a large and successful groups which belongs to the order of Blattodea. They are widely distributed throughout tropical, subtropical and temperate regions of the world (Eggleton, 2000). Furthermore, they are found abundantly in temperate areas, where their biomass was almost similar to tropical termites (Gentry and Whitford, 1982; Bignell and Eggleton, 2000). In Malaysia, termite are known as ones of the most important pest. *Coptotermes gestroi* and *Globitermes sulphureus* are among the most common termite species found in Malaysia (Lee et al., 2007). The genus of *Coptotermes* spreads widely in tropical and subtropical region of the world whereas *G. sulphureus* is heavily found in Indo-Malayan region (Abdul Hafiz et al., 2007). Termites are a very important agents in nutrient recycling (Gosling et al., 2012). Besides, they are important as a major decomposers of organic matter (Bignell and Eggleton, 2000). Paradoxically, termites become an important pest when they are being introduced to man modified environments such as houses, buildings and cultivated crops (Evans et al., 2013). Although termites have a soft and fragile body, their hard and soothed jaws are capable to bite off wood and some termite species can destroy living plants within a short period of time (Mueller and Gerardo, 2002). Subterranean termites, *C. fomosanus* and *C. gestroi* are the most serious pest insects that can cause substantial economic loss as high as $32 billion in the world (Rust and Su, 2012).

Synthetic insecticides have been used in a long time for termite control management. Soil treatments, baiting and dusting have been relied on to control termites

(Lee et al., 2003). However, Wako (2015) stated that the application of chemical insecticide may be effective to certain extents but they are not highly recommended due to their harmful effect to environment.

Nowadays, society are more interested in natural insecticide by using plant extract than commercial pesticide due to the concern of public health and environment conditions (Duke et al., 2003). The research for natural insecticide requires the screening of naturally occurring bioactive compounds in plants (Isman, 2006). They need to be screened in order to identify their activities and the active molecules should be characterized (Gonzalez-Coloma et al., 2010). Plants produce an incredible array of secondary metabolites. Secondary metabolites were once thought to be waste compounds (Raven et al., 2005). However, understanding on the function of secondary metabolites are gradually increasing as many studies have been conducted. Secondary metabolites which derived from plant such as alkaloids, steroids, terpenoids, essential oils and phenolics are reported for having the insecticides activities (Ghosh et al., 2012). Many plants have been developed into economically important products including nicotine, rotenone, ryania, sabadilla and pyrethrum (Arnason et al., 2010). Among the group of secondary metabolites, plant phenolic compounds are a large group that affect the larval growth and development of herbivores by inhibit their feeding activity or, in post-ingestive phenomena (Treutter, 2006). Flavonoid compounds such as rutin and quercetin are abundantly found in vegetables, fruits, herbs, leaves and seeds (Harborne, 1986; Havsteen, 1983). Rutin and quercetin are reported for having anti-inflamammatory, analgesic and antioxidant activity (Di Carlo et al., 1999; Emim et al., 1994; Morand et al., 2000; Silva et al., 2016). Rutin is a bioflavonoid that protect plant against insects due to their anti-nutritional effect (Silva et al., 2016).

Unlike chemical insecticides that are based on a single ingredient, natural plant insecticides have an array of several compounds that decrease the possibility of insects to develop resistance (Koul and Walia, 2009). Plant extracts with complex mixtures of chemical compounds were tested for their insecticide, repellent and devoured properties (Isman, 2006; Nisar et al., 2012). Plant natural insecticides mostly work as repellents which drive the insects away from the crops by their smell or taste, oviposition which prevent the insect lying their eggs, antifeedants and inhibitors which promote the suppression of calling behaviour and growth (Sutherland, 1977). In addition, many plants have been recognized to have anti-termitic activities or known as repellent to termites (Sakasegawa et al., 2003). From previous studies, many researchers tested plant extract against termites (Sakasegawa et al., 2003; Raina et al., 2007; Oyedokun et al., 2011; Pandey et al., 2014).

The followings are the objectives of this study.

Study 1 (Chapter 3): Evaluation of the toxicity and repellency effect of selected plants extracts on *Coptotermes gestroi* and *Globitermes sulphureus.*

Aim:

1. Determination of different solvents to extract the termiticides through no choice bioassay at different concentrations.
2. Determination of repellency activity of plant extracts with different solvents through choice bioassay at different concentrations.
3. Determination of termite mortality with different flavonoids through no choice bioassay at 10 000 ppm.

Study 2 (Chapter 4): Evaluation of the efficacy of the plant extracts on the tunneling and surviving of *Coptotermes gestroi* and *Globitermes sulphureus.*

Aim:

1. Determination of termite tunneling activity at different concentrations.
2. Determination of termite behavior after applying the extracts through tunneling bioassay.

Study 3 (Chapter 5): Identification of the bioactive compounds from methanolic plant extracts.

Aim:

1. Identification of the plant compounds in bioactive extracts by using Gas Chromatography Mass Spectrometer (GC-MS) and phytochemical test.

CHAPTER 2

LITERATURE REVIEW

2.1 General introduction

Termites (Order: Blattodae) are social insects that compromising less than 3105 species in the world with about 185 from described species of termites considered to be pests (Krishna et al.,2013). They are known as social cockroaches and no longer being classified as order Isoptera due to the close phylogenetic relationship between termites and *Cryptocercus* (Inward et al., 2007). Termite becomes economic pests when they consume wooden structures, flooring, clothing, books and household furniture (Prahlad and Chimkod, 2012; Wako, 2015). Family of Kalotermitidae and Rhinotermitidae are invasive species as they consume wood as food source, nest in food, and easily produce secondary metabolites (Evans et al., 2013). The subterranean termite, *Coptotermes* spp is the most damaging pest that infested almost 85% of premises in Northern Peninsular Malaysia (Lee et al., 2007).

Biological control constitutes are more environmentally friendly compared to chemical control. In plant, pesticidal compounds exist within almost all classes of secondary metabolite (Miresmailli and Isman, 2014). Secondary metabolites act quickly, degrade rapidly and have, with a few exceptions, low mammalian toxicity (El-Wakeil, 2013). With the general public becoming increasingly concerned about pesticide usage, the use of bio-control to control termite is a potentially promising market (Kuswanto et al., 2015).

2.2 Subterranean termite biology

Termites are living in large colonies in a variety of nests (Ghaly and Edwards, 2011). They have a cryptic nature and very small in size, yet their numbers of individual can reach up to millions in a colony (Abdul Hafiz and Abu Hassan., 2008). Their bodies are soft and pale in color with the presence of mouth part for biting, chewing and utilizing the cellulose as the food source (Verma et al., 2009). Body structure of termites consists of a thorax that joined broadly to the abdomen with wide waist (Grimaldi and Engel, 2005). Termites undergo incomplete metamorphosis with egg, nymph and adult stages (Prahlad and Chimkod, 2012). In a typical termite colony, it consists of nymph alates, workers with pale-colored heads, soldiers with red-colored heads and reproductive individuals of both sexes (Wako, 2015).

Lee and lee (2012) reported in the termite colony, worker constituted the largest proportion (42.19%) of total number, followed by larvae (42.60%), soldiers (14.16%), pre-soldier (0.71%) and alate (0.34%). The colony of termites consists of a set ratio of soldiers to workers and nymphs (Wako, 2015). Any extra number of soldiers will cause trouble and burden to the colony because they must be feed by workers member (Kuswanto et al., 2015). In addition, additional members will be developed from nymphs if members of any caste are lost in order to restore the balance and number of termite individuals in caste of colony (Wako, 2015). Termite caste can be divided into two types of individuals which is fertile (reproductive) and sterile individuals (Roisin, 2000). The sterile castes include workers, pre-soldiers and soldiers while alates, primary reproductive and secondary reproductives are fertile (Watanabe et al., 2014). Termite workers and soldiers are wingless and lack of eyes (Lee and Neoh, 2014). They are quite small with 6 mm long, and when compare to the workers, soldiers have enlarged head with noticeable jaws that eject liquids (Verma et al., 2009; Prahlad and Chimkod, 2012). Termite workers perform various tasks including feeding the other caste, grooming the loyals, making tunnels and excavating the nest (Lee and Neoh, 2014), while termites soldiers are responsible for guarding the colony and its occupants (Ghaly and Edwards, 2011). Besides, termite soldiers may be involved in exploration of new food source like *Heterotermes tenuis* (Casarin et al., 2008). Alates have wings, tested and ovaries, and they are developed from nymphal stages (Watanabe et al., 2014). The primary reproductive (king and queen) key roles is production of eggs and distribution by colonizing flights (Verma et al., 2009), while secondary reproductive are responsible to substitute primary reproductive when the queen becomes old or dies (Lee and Neoh, 2014). Queens lay thousands of eggs a day through her enlarged abdomen (Prahlad and Chimkod, 2012).

Termites live in a large colonies (Plate 2.1). During the swarming seasons, alates emerge in a mass nuptial flight. This flight showed the first indication of termite infestation. Once alates leave their nest to find the new nesting sites, they will land a few hundred meter away (Ghaly and Edwards, 2011). Lee and Neoh (2014) stated that their flight can reach a height of about 15 m with a distance of 200-300 m. Their flight distance may be improved with the help of winds and air currents. After the flight, alates paired, shed their wings and female alates immediately search the nesting site with males follows closely behind (Verma et al., 2009). When the pair succeed at finding the new site, they form the royal chamber and seal it (Prahlad and Chimkod, 2012). The queen starts laying yellowish white eggs and hatch after 50-60 days (Wako, 2015). Freshly hatched young ones are called as larvae, and they grow without significant morphological changes

(Wako, 2015). However, due to their soft and incomplete shape mouthpart, larvae must be fed by both king and queen until they are able to feed themselves (Lee and Neoh, 2014). Larvae develop into workers or nymph after a several moults (Ghaly and Edwards, 2011). The newly form workers will help the king and queen by collecting their own food, care the eggs and young larvae, and enlarge the nest (Lee and Neoh, 2014). Nymphs develop either into alates or neotenic reproductive (Vargo and Husseneder, 2009). However, nymph will be produced after the colony is mature and food is abundant (Lee and Neoh, 2014). Soldiers are derived from all worker instars, pseudorgated or apterous immature forms (Casarin et al., 2008). However, unlike other caster, soldier unable to molt (Lee and Neoh, 2014).

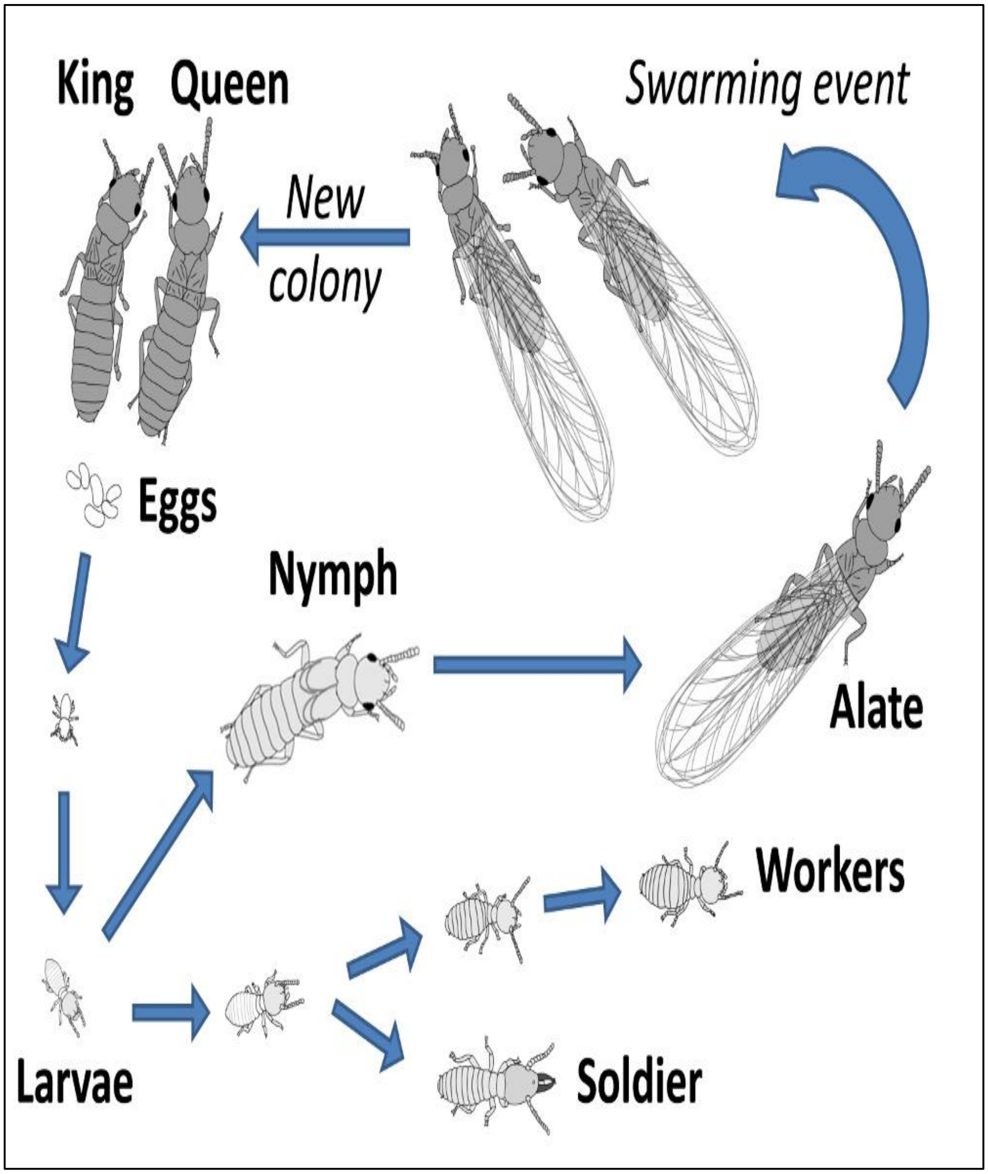

Plate 2.1 life cycle of termites. *Coptotermes gestroi*, retrieved on 17 November, 2016 from http://flrec.ifas.ufl.edu/termites-in-florida/life-cycle/

2.3 Evolutionary status into lower and higher termites

Termites are divided into seven major families, which one of these families was known as higher termite and the remaining six families represented the lower termites (Abe et al., 2002; Ottesen and Leadbetter, 2011). The higher termites are a single family of Termitidae which dominating 85% of the genera (Rahman and Tawatao, 2003). On the contrary, the lower termite families which constitute 25% of genera were known as Mastotermitidae, Klotermitidae, Hodotermitidae, Termopsidae and Rhinotermitidae (Lee et al., 2014).

Lower termites house a diverse population of flagellated protist (unicellular eukaryoyes), bacteria and archaea in their hindgut (Peterson and Scharf, 2016), whereas the higher termite typically lack the flagellated protist (Ohkuma, 2003). Lower termites were depended on wood or grass as their food source (Ottesen and Leadbetter, 2011). They actually depend on their symbotic intestinal protozoa for cellulose digestion (Lee et al., 2014). On contrary, higher termites showed variation in their feeding behavior including wood, soil for nutrition and cellulytic fungi (Ohkuma, 2003). Because of their lack of symbiotic protozoa, they rely on the action of endogenous enzymes from bacteria and symbiotic fungus (Wood and Thomas, 1989). In addition, higher termites is a major group in tropical forest system as litter, wood and soil feeders, and lower termites are mostly wood feeders which usually predominate outside forests or in marginal habitats within forests (Rahman and Tawatao, 2003).

2.4 Termite infestation and economic loss in worldwide and Malaysia

Termites play an important role in soil ecosystem and found throughout the world (Ghaly and Edwards, 2011). They can be found in tropical and subtropical regions and represent in about 10% of animal biomass (Donovan et al., 2007). Termites help to improve and maintain the physical, chemical and qualities of soil (Dawes, 2010). They also play key roles as recyclers of up to 20% of dead wood in tropical ecosystems (Bignell and Eggleton, 2000). Termites feed on dry grass, decaying leaves, animal dung, humus and living or dead wood (Brossard et al., 2007). However, they become a major problem when introduced to urban areas as they give negative economic effects, devaluing property, damaging crops and necessitating household repair (Ghaly and Edwards, 2011). Kuswanto et al. (2015) stated that termite attacks on building or structures are initiated from a nest in a ground and eventually build several tunnel galleries on trees or walls of the structures to reach cellulose. Sometimes, these tunnel with tens to hundreds meters in length will connect multiple feeding sites (Arab et al., 2012). Next, termites will deposited trail pheromones from sternal gland on substrate for orientation and recruitment of workers and soldiers toward the food source (Arab and Costa-Leonardo, 2005). When they are inside the building, they continuously in contact with the ground and nest center for moisture and communication purposes (Kuswanto et al., 2015).

Subterranean termites attack against structures or buildings mainly occur in areas formerly forested land or plantation (Mo et al., 2006). Human activities like land clearing of forest or plantation are usually left stump and piles of litter scattered on or in the ground (Kuswanto et al., 2015). Generally, pest are responsible in building damage and causing a high cost of termite prevention that may reach upon billions (Ahmed and French, 2005).

The global damage caused by termites was estimated at US $ 22 billion to US $ 40 billion worldwide (Rust and Su, 2012). Lee et al. (2007) reported that the cost of damage in Southeast Asia was estimated at US $ 200 million per year. In addition, subterranean termites cause 90% of the total of economic loss and about 70% of structure damages (Kuswanto et al., 2015). Common termite genera that usually attack buildings and structures in Malaysia are *Coptotermes*, *Schedorhinotermes*, *Microtermes*, *Macrotermes*, *Nasutermes*, *Globitermes* and *Odondotermes* (Lee et al., 2007). *Coptotermes* sp is the most destructive termite species and infested nearly 85% premises in Northern Peninsular Malaysia (Lee et al., 2007). *C. gestroi* often can be found infesting buildings and structures whereas *C. curvignathus* is a common pest in premises built on ex-agricultural land or plantations, especially rubber, oil palm and coconuts (Lee et al., 2007). Besides, *G. sulphureus* can cause severe damage to coconut and oil palm plantations and surprisingly becomes a secondary pest species after the elimination of *Coptotermes* sp with baits (Lee and Neoh, 2014).

2.5 Termites control and management

Various control measures are used to prevent and control termite infestation, including physical, chemical and biological control. However, chemical pesticide commonly has been used over the years for termite control (Kuswanto et al., 2015).

A number of alternatives to using chemical pesticides for termite control such as baiting system and soil treatment. In recent years, baiting system is a more popular option to control the subterranean termites (Lee et al., 2014). Baits and wood treatments depend on foraging termites to encounter, feed on and horizontally transfer residual insecticide deposits (Scharf, 2015). Baiting system is a promising method which at least targeting for colony elimination (Evans, 2010). There are two types of termite baiting products that are in-ground (IG) and above-ground (AG) bait system. IG bait system is installed in the soil surrounding a structure to intercept foraging termites and AG bait system usually comprises a bait box with one open side mounted on a wall that is infested with termites (Su, 2015). In this treatment, it only required a small amounts of toxicant compared to soil barrier treatment and the toxicant is very specific to targeted pest and have low mammalian toxicity (Broadbent, 2011). The toxicant in bait containing chitin synthesis inhibitors (CSIs) can interfere the formation of chitin during molting process, which targeting the immature stages such as larvae, nymphs and workers (Neoh et al., 2011). There were many studies related to the successful elimination to lower termites by baiting system (Said et al., 2000; Xing et al., 2005; Gautam and Henderson, 2014). However, there were relatively few studies documented the effectiveness of this bait against higher termites (Neoh et al., 2011; Lee et al., 2014). Moreover, Neoh et al. (2011) stated that the time required to eliminate higher termite colony, *G. sulphureus* was more than 4 months longer than required for lower termites (Neoh et al., 2011).

In addition, soil treatment was applied in order to build an insecticide barrier around building structures and protect them from termite attack (Buczkowski et al., 2012), thus they perceived to be simpler, safer and more persistent (Evans and Iqbal, 2015). This treatment depends on the physical contact between termites and insecticide residues (Scharf, 2015). In this treatment, the most common liquid termiticide used are non-repellent and can cause delayed termite mortality (Baker and Miguelena, 2014). Slow acting action also allows termites to penetrate in treated soils for considerable periods before they becomes intoxicated, immobilized and eventually die (Saran and Rust, 2007). Before the treated termite died from the toxicant, they have sufficient time and opportunity to interact with thereby contaminate the untreated nestmates in locations that beyond the areas where they are originally applied (Quarcoo et al., 2012; Baker and Miguelena, 2014). In the termite control industry, fipronil is most commonly used non-repellent compounds (Saran and Kamble, 2008).

2.6 Limitation of using chemical insecticide

The overuse of pesticide in quantity and frequency application lead to a major problem to the targeted pest which causing them to disperse to new environment or adapt to the novel conditions (Cothran et al., 2013). The adaptation of the targeted pest to the new environment might be due to the gene mutation, change in population growth rates and increase in number of generation (Gill and Garg, 2014). Therefore, this has resulted in resurgence and appearance of targeted pest that are resistant to pesticide (Gill and Garg, 2014).

In addition, pesticide also has resulted in serious health problem to man and may be harmful to the environment (Verma et al., 2009). According to Aktar et al. (2009), exposure of pesticide to the production workers, formulators, sprayers, mixer, loaders and agricultural farm workers might be high. Pesticide are persistence in the environment, and they are able to enter the human body through direct contact with chemicals, food products especially fruits and vegetables, through contaminated air and water (Gill and Garg, 2014). Toxic effect such as irritation and other allergic symptoms can result from pesticide exposure (Damalas and Koutroubas, 2016). Severe symptoms like strong headache, dizziness, or nausea also probably to be detected (Damalas and Koutroubas, 2016).

In the past, organochlorine insecticide was the effective way to control termite infestation (Pearce, 1997). However, organochlorines were banned as they are insoluble, persist in soil and aquatic sediments, can bio-concentrate in the tissues of invertebrates and vertebrate from their food, move up in trophic chains and affect top predators (Sarwar, 2016). Gavrilescu (2005) stated that pesticide still can be detected in surface and groundwater despite some of them were already banned and replace by environmental friendly substitute. In addition, some of the most potent insecticide used to treat termites such as aldrin and dieldrin also were no longer available in the commercial market as they are highly expensive, have harmful effects on human health and non-target organism, environmental pollution and termites have been reported to have developed resistance towards these insecticides (Sileshi et al., 2009).

2.7 *Coptotermes gestroi*

Kingdom	: Animalia
Family	: Rhinotermitidae
Genus	: *Coptotermes*
Scientific name	: *Coptotermes gestroi*

C. gestroi was known as important pest, which primarily found in tropical regions (Li et al., 2009). Asian countries including the Philippines, Malaysia, Singapore, and Indonesia were found to be the center of origin for *C. gestroi* (Li et al., 2009). The lower termite, *C. gestroi* was found to be attacking buildings in urban area, rural or suburban area (Kirton and Azmi, 2005). This species is well adapted to those environments where human settlements prevail and it attacks mainly wooden structures like cabinets, parquet floors, windows, door frames and roofs.

C. gestroi has a main nest connected with secondary nest (Janei and Costa-Leonardo, 2015). The main nest houses the imaginal king and queen, while the secondary nest was established because of the growth of the colony. The workers are numerous and largest populated caste in the colony (Janei and Costa-Leonardo, 2015). Their soldiers have one pair of hairs near the rim of the fontanelle (Scheffrahn and Su, 2008). They secrete a white liquid from a fontanelle as a defensive secretion (Wikipedia contributors, 2016).

2.8 Globitermes sulphureus

Kingdom	: Animalia
Family	: Termitidae
Genus	: *Globitermes*
Scientific name	: *Globitermes sulphureus*

G. sulphureus is highly distributed in the Indo-Malayan region, from Myanmar to peninsular Malaysia (Tho, 1992) ( ). Their mound with a dome-shape earthen can be up to 80 cm in height and 60 cm in diameter (Neoh et al., 2011). The soldier of this species is the only one in Peninsular Malaysia that have a very bright yellow body in which the salivary gland extends to the end of abdomen (Ngee and Lee, 2002; Tho, 1992). The other unique characteristic is the termite soldiers secrete a yellow liquid which entangles both termites and their enemies while attacking (Bordereau et al., 1997). They also have rounded head, strong incurved mandible with prominent tooth on the inner edge and the salivary gland extend to abdominal cavity (Tho, 1992).

G. sulphureus attack agricultural crops such as rubber, coconut, oil palm and sugar cane plantations (Tho, 1992). Sometimes, they can be found in building structures in rural and suburban areas (Kirton and Azmi, 2005). They are also well known because of its secondary pest (Neoh et al., 2011). From the previous study, this species were found in premises that were treated with bait after the previous infested termites, *C. gestroi* has been eliminated (Lee et al., 2007). This species was found infesting door and window frames of dwelling (Ngee and Lee, 2002).

2.9 The uses of plants natural insecticide

The development and establishment of plant derived pesticides are called as botanical pesticide, biopesticide or natural pesticides (Copping and Menn, 2000). Plant derived pesticides, or also known as botanicals comprise of crude extracts and isolated or purified compounds from numerous species of plants and commercial products (Siegwart et al., 2015). Plant pesticides compose of various advantageous properties such as insecticidal activity, repellency activity to pests, deterrent to feeding, insect growth regulation and toxicity to insect pests (Sharma and Gupta, 2011). Up to now, more than 2000 species of plants were recognized to possess the insecticidal activity, where some of them belong to several plant families includes Asteraceae, Euphorbiaceae, Fabaceae, Labiatae, Meliaceae and Solanaceae (Castillo-Sánchez et al., 2010).

Plant pesticides or plant derivatives were very popular and used in agriculture areas since two millennia in ancient Egypt, China, Greece and India (Thacker, 2002; Ware, 1983) Besides, the uses of plant pesticides in Europe and North America were reported back more than 150 years (El-Wakeil, 2013). Amongst the earliest natural pesticides were nicotine, rotenone and pyrethrum which extracted from tobacco, derris root and chrysanthemum (Ballantyne and Marrs, 2004). However, they had several weaknesses where pyrethrum was not stable in sunlight and has short lived, rotenone was found to be toxic to fish and nicotine was banned due to high mammalian toxicity (Capinera, 2008). Reported by Capinera (2008), plant pesticides that currently used for insect suppression include neem from the seeds of *Azadirachta indica*, rotenone, pyrethrum and essential oils. Several plant pesticides include ryania, nicotine and sabadilla were also used for insect control but only in limited use (El-Wakeil, 2013).

According to Dayan et al. (2009), latest reports indicate that sales of organophosphates are decreasing, while natural products and natural product-derived insecticides are continually increasing. In the worldwide sales, three out of five major insecticides are natural products or natural product-derived insecticides (Nauen, 2006).

Pesticides that derived from plant can be grouped according to their mode of action or the way they destroy or control the target pest (El-Wakeil, 2013). According to Belmain et al. (2001), plant pesticide is safer to environment, easily processed, less expensive and used by farmers and small industries. Generally, natural insecticide will not targeting to the non-target organism and very specific (Elango et al., 2012). Moreover, natural insecticide derived from plants do not develop resistance in pests and pathogen due to the presence of various active compounds (Pavela, 2009). The redundancy of active compounds was also able to increase the performance of plant extracts through analog synergism as well (Arnason et al., 2010). Interestingly, natural insecticide does not persist in the environment in a long time and degrade rapidly, as they have a short half-life because they do not have ‘unnatural’ ring structure and possess relatively few halogen substituents (Dayan et al., 2009). The rapid degradation may be advantageous as it lessens the risk of residues on food (El-Wakeil, 2013). For instance, pyrethroids and pyrethrins extracted from *Chrysanthemum* genus have a short half-life in the environment (Schleier and Peterson, 2011). Piperamides and alpha terthienyl demonstrated that they are able to degraded in the environment in hours or days (Arnason et al., 2010).

2.10 Plant secondary metabolites

Screening of the natural products has gained the attention worldwide among the researchers (Kebede et al., 2010). Phytochemicals are divided into primary metabolites and secondary metabolites based on the function in plant metabolism (Saxena et al., 2013). Primary metabolites are valuable for the growth and development of plant (Hong et al., 2016). The example of primary metabolites include phytosterols, acyl lipids, nucleotides, amino acids, proteins, carbohydrates, lipids and nucleic acids, are present in all plants (D'Incao et al., 2013; Hussain et al., 2012). On contrary, secondary metabolites has no role in the growth and development of the plant (Hussain et al., 2012). Secondary metabolites have been acquired from glucose metabolism via ethyl acetate and shikimic acids pathway (D'Incao et al., 2013). They are highly concentrate, accumulated in a smaller quantities than primary metabolites, and even restricted in within and between species (Irchhaiya et al., 2015; Moore, 2002). Unlike primary metabolite, plant produces secondary metabolites in response to specific environmental stimuli for survival (Hong et al., 2016). For instance, the damage or attack from herbivore, attack from pathogens and nutrition depravation (Hermsmeier et al., 2001; Reymond et al., 2000). They are also well known for having the insecticidal properties (López et al., 2008). Originally, plant was used as home remedy to kill and repel insects (Kim et al., 2010). The existence of pesticidal compounds were found in almost of secondary metabolite classes (Malesevic et al., 2015). The classifications of plant secondary metabolites are based on the biosynthetic pathway (Harborne et al., 1999). Secondary metabolites divided into three largest groups which are terpenoids, phenolic compounds, and nitrogen-containing compounds/alkaloids (Irchhaiya et al., 2015).