LABORATORY EVALUATIONS OF SOME TERMITICIDES AGAINST SUBTERRANEAN TERMITE *Coptotermes gestroi* (Wasmann) (Isoptera: Rhinotermitidae)

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2008

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by

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Thesis submitted in fulfillment of the Requirements for the degree Of Master of Science

July 2008

ACKNOWLEDGEMENTS

Firstly, and foremost praise be to ALLAH the Great and Almighty surrounded me under his auspices during my master study, in the School of Biological Sciences, Universiti Sains Malaysia.

I would like to express my sincere gratitude to my supervisor Professor Abu Hassan Ahmad, the Dean of School of Biological Sciences, Universiti Sains Malaysia, for his advice, invaluable guidance and comments during experimental work and thesis writing, not just limited to my master degree but also for the three years of supervision and friendship in the Medical Entomology Laboratory, School of Biological Sciences, Universiti Sains Malaysia.

I am also grateful to Professor Lee Chow Yang, in School of Biological Sciences, Universiti Sains Malaysia, for his advice, knowledge and helps for identification of the termite. Special thanks are extended to Professor Bahruddin bin Saad in the School of Chemical Sciences, Universiti Sains Malaysia, for his helps in the HPLC analysis.

Sincere thanks to my friend Abdul Hafiz B. Ab Majid for helping me in the laboratory during my study and friendship, and for helping me to collect the termites needed for my experiments. I cannot forget the three years in Medical Entomology Laboratory with him.

I also extend special thanks to my friend PhD candidate Mohammed Al douh from the School of Chemical Sains, Universiti Sains Malaysia for helping and teaching me how to use the UV Instrument and for all the joyfulness. Additionally, I also thank PhD candidate Mr. Ahmad Al Makahelah, Miss. Mardiana and Mr. Arefeen from the

School of Chemical Sciences, Universiti Sains Malaysia for teaching me how to use the HPLC.

Thanks also to Mr. Mohad Hadzri, Mr. Adnan and Muthu for technical assistance, and the Vector Control Research Unit, Universiti Sains Malaysia for helping me in my study.

I wish to thank my friends Dr. Hassan Obaid Al Fadly, Dr. Seed Obaid Al Fadly and PhD candidate Rais Abdulqader Attamimi for correcting my thesis in the English language, and their advice in academic writting.

I am very indebted to my close friend Dr. Yaser Abdulqader Al jahory for everything he has done for me since my arrival in Malaysia until I finished my study. I would like to express my thanks to Dr. Makarum Mohammed Ba Matraf for his kindness.

Finally great thanks to my mother (Zahrah), father (Salem), sisters (Fatemah, Dekrah, Enas) and brothers (Mohammed, Abdullah, Anees). I wish to express my special thanks to my wife (Saba) and my baby daughter (Aesha) for every thing that helped me to complete my study.

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LIST OF ABBREVIATIONS

The following abbreviations have been used commonly throughout this thesis

A = (Agenda) Trade name of Fipronil used.

Donor termite = Termites treated by termiticide directly.

EC = Emulsifiable concentration.

HPLC = High performance liquid chromatography.

LC50 = Lethal concentration to kill 50 percent of test population.

LC90 = Lethal concentration to kill 90 percent of test population.

LENTRAK = Trade name of chlopyrifos used.

LT50 = Lethal time to kill 50 percent of test population

LT90 = Lethal time to kill 90 percent of test population

mg = milligram

min = minutes

ml = milliliter

MS = Mean sequare

Nile blue A = Chemical material used for marking termites.

P value = statistics indicator to test null hypothesis.

Pinus *cariba* = Species of wood used to feed termites.

ppm = Part per million.

Premise 200 SC = Trading name of Imidacloprid used.

Regent 50S = Trade name of Fipronil used.

r value = statistics value to determine the relationship between the factors

Recipient termite = Termite receive toxicity by donor termite

SC = Suspension concentration

S.D = Standard deviation

S.E = standard error

Steward = Trade name of Indoxacarb

UV= Ultraviolet

W1 = first weight of filter paper before feeding

W2 = Second weight after feeding

W /w = Weight by weight

PENILAIAN-PENILAIAN MAKMAL BEBERAPA RACUN ANAI-ANAI KE ATAS ANAI-ANAI BAWAH TANAH *Coptotermes gestroi* (Wasmann) (Isoptera: Rhinotermitidae)

ABSTRAK

Kesan bagi lima jenis racun anai-anai dinilai di dalam makmal, ke atas *C. gestroi* untuk menentukan kesesuaiannya di dalam mengawal anai-anai bawah tanah. Ujian pemakanan– paksaan, ujian pemakanan–pilihan, kesan pindah dan kedalaman kemasukan dalam tanah telah digunakan untuk menilai racun anai-anai. Lima racun anai-anai telah digunakan dalam kajian, Premise® SC 200 (kandungan aktif: Imidacloprid 18.3% wt/wt), Agenda™ 2.5 EC (kandungan aktif: Fipronil 2.92 % wt/wt), Regent 50 SC (kandungan aktif : Fipronil 50g), Steward (kandungan aktif : Indoxacarb 14.5 % wt/wt) adalah racun anai-anai yang tidak bersifat mengusir anai-anai. Manakala, Lentrek 400 EC (kandungan aktif : Chlorpyrifos 38.7% wt/wt) adalah racun yang bersifat mengusir anai-anai.

Racun yang mengusir (Chlorpyrifos) menyebabkan banyak kematian ke atas *C. gestroi*, manakala yang bersifat tidak mengusir (Imidacloprid, Fipronil dan Indoxacarb) menyebabkan kematian yang boleh diterima ke atas anai-anai, terutama sekali di dalam kepekatan rendah dalam lingkungan 24jam. Nilai LC_{50} bagi Chlorpyrifos adalah 0.8ppm, manakala LC_{50} untuk racun anai-anai yang tidak mengusir adalah 392ppm, 5626ppm, 2747ppm dan 858ppm bagi Imidacloprid, Fipronil (Agenda), Fipronil (Regent) dan Indoxacarb masing-masingnya.

Imidacloprid adalah berkesan terhadap *C. gestroi* diikuti oleh Indoxacarb, Fipronil (A) dan Fipronil (R). Racun anai-anai Chlorpyrifos, menampakkan kematian yang lebih tinggi pada hari pertama dan setelah itu, ia menjadi pengusir kepada anai-

anai. *Coptotermes gestroi* masih terus memakan kertas turas yang telah dimasukkan dengan racun anai-anai yang tidak bersifat mengusir, mencadangkan yang racun anai-anai ini boleh digunakan secara berkesan sebagai umpan dalam mengawal (anai-anai bawah tanah) *C. gestroi*. Kajian ini menunjukkan kesan pindah racun yang tidak mengusir anai-anai, dari anai-anai penyumbang kepada anai-anai penerima. Kadar purata kematian bagi anai-anai penerima adalah di antara 0.35 ke 16.05 bagi Imidacloprid, 4.5 ke 39.6 bagi Fipronil, dan 4.2 ke 15.75 bagi Indoxacarb. Manakala kadar purata kematian bagi anai-anai penyumbang adalah dalam lingkungan 0.7 ke 2.5, 1.8 ke 4.75 dan 0.85 ke 2.95 bagi Imidacloprid, Fipronil dan Indoxacarb masing-masingnya.

Kesan racun anai-anai yang bersifat mengusir dan tidak mengusir ke atas kemasukan *C. gestroi* ke dalam tanah adalah signifikan untuk kesemua racun anai-anai kecuali Indoxacarb. Tidak ada perbezaan signifikan antara kepekatan Indoxacarb dan kemasukan ke dalam tanah, ini bererti kepekatan Indoxacarb tidak mempengaruhi keupayaan *C. gestroi* untuk masuk ke dalam tanah yang telah di rawat. Juga, terdapat suatu perbezaan signifikan antara ketebalan tanah yang dirawat dengan racun anai-anai dengan kemasukan anai-anai, kecuali untuk Indoxacarb (MS =100.895, F = 1.067, P = 0.372).

Kepekatan Chlorpyrifos, mempengaruhi secara signifikan kemasukan *C. gestroi* ke dalam tanah (MS = 10.277, F = 1008.932, P = 0.0001). Ketebalan tanah yang dirawat dengan Chlorpyrifos juga mempengaruhi secara signifikan kemasukan anai-anai (MS = 0.178, F = 17.47, P = 0.0001). *Coptotermes gestroi* berjaya memasuki ≥ 50% dari 1cm tanah yang dirawat dengan 0.001, 1.0, 10 ppm Chlorpyrifos dan kurang dari 40% ketebalan tanah yang dirawat dengan100 dan 1000 ppm Chlorpyrifos.

LABORATORY EVALUATIONS OF SOME TERMITICIDES AGAINST SUBTERRANEAN TERMITE *Coptotermes gestroi* (Wasmann) (Isoptera: Rhinotermitidae)

ABSTRACT

The effects of five termiticides were evaluated in the laboratory against *C. gestroi* to determine their efficiency in controling subterranean termites. Force-feeding test, choice-feeding test, transfer effect and soil penetration were used to evaluate the termiticides. Five termiticides were used, in this study, Premise® SC 200 (active ingredient: Imidacloprid 18.3% wt/wt), Agenda™ 2.5 EC (active ingredient: Fipronil 2.92 % wt/wt), Regent 50 SC (active ingredient: Fipronil 50g), Steward (active ingredient: Indoxacarb 14.5 % wt/wt) were the non-repellent termiticides. While, Lentrek 400 EC (Active ingredient: Chlorpyrifos 38.7% wt/wt) is the repellent termiticide.

The repellent termiticide (Chlorpyrifos) caused high mortality on *C. gestroi*, while non repellent termiticide (Imidacloprid, Fipronil and Indoxacarb) caused acceptable mortality on termites, particularly in low concentrations within 24hours. The LC_{50} value of Chlorpyrifos was at 0.8ppm, where as the LC_{50} of non repellent termiticides were 392ppm, 5626ppm, 2747ppm and 858ppm for Imidacloprid, Fipronil (Agenda), Fipronil (Regent) and Indoxacarb, respectively.

Imidacloprid was effective on *C. gestroi* followed by Indoxacarb, Fipronil (A) and Fipronil (R). Chlorpyrifos termiticide, showed higher mortality on the first day and then it became a repellent to the termites. *Coptotermes gestroi* continued to feed on filter paper impregnated with non repellent termiticides, suggesting that these termiticides can

be used effectively as bait for controlling (subterranean termite) *C. gestroi*. This study showed the transfer effect of non repellent termiticides from donor termites to recipient termites. The mean mortality of the recipient termites ranged from 0.35 to 16.05 for Imidacloprid, 4.5 to 39.6 for Fipronil and 4.2 to 15.75 for Indoxacarb. While the mean mortality of the donor termites ranged from 0.7 to 2.5, 1.8 to 4.75 and 0.85 to 2.95 for Imidacloprid, Fipronil and Indoxacarb, respectively.

The effect of repellent and non repellent termiticides on *C. gestroi* penetration was significant for all termiticides except Indoxacarb. There were no significant differences between Indoxacarb concentrations and soil penetration, which means that Indoxacarb concentrations did not affect *C. gestroi* ability to penetrate the treated soil. There was also a significant difference between the soil thickness treated with termiticide and termite penetration except for Indoxacarb (MS =100.895, $F = 1.067$, $P = 0.372$). Chlorpyrifos concentrations significantly affected *C. gestroi* s penetration (MS = 10.277, $F = 1008.932$, $P = 0.0001$). The thickness of the soil treated with Chlorpyrifos also significantly affected the termites penetration (MS = 0.178, $F = 17.47$, $P = 0.0001$). *Coptotermes gestroi* successfully penetrated ≥ 50% of 1cm soil treated by 0.001, 1.0, 10 ppm of Chlorpyrifos and less than 40% of the treated soil thickness for 100 and 1000 ppm

CHAPTER 1

1.1. GENERAL INTRODUCTION

Termites have been reported worldwide as one of the most important group of insects that cause significant and serious damages to crops, structures and buildings (Harrris, 1969; Harris, 1971; Hickin, 1971; Tho and Kirton, 1990; Khoo et al., 1991; Tho, 1992; Pearce, 1997; Sajap et al., 1997; Baskaran et al., 1999 Sajap, 1999; Su and Scheffrahn, 2000; Gurbel, 2002; Lee et al., 2003b; Lee and Chung, 2003a). Around US$ 22 billion are spent annually for termite control and repairing the damages (Su, 2003). In Japan, several hundred million dollars were spent annually for the prevention and control of termites (Kubota et al., 2006). Surprisingly, that is much more higher than the cost of repairing the total damages caused by the natural disasters such as fires, earthquakes, tornadoes (Hedges,1992).

Lee et al. (2003) stated that the termite infestation is a serious major problem in many tropical countries. Termite damage to historical buildings is both costly and irreversible and diminishes the integrity of a structure (Su et al., 1998). In Australia, it has been reported that there is only 20 species which are conducive a significant damage to timber-in-service structures and ranged between 0.9 to 4.5% per year (Peters, 1996). Ngee et al., (2004) reported that the cost of termite control in Malaysia was estimated to be US $10-12 million in 2003.

Among different genera of termites, *Coptotermes* has been reported as responsible for more than 90% of the total infestation in buildings and structures in West Malaysia (Lee 2002b; Lee and Chung, 2003).

1.2. The damage to wooden structures

Termites consume about 7 billion tons of biomass, mainly wood and other forest litter each year. Only 5% of termite species are recognized to cause serious damages to timber-in-service and wooden structures globally (Ahmed, 2000). In USA For instance, many studies emphasized on the importance of termite control which cause significant destruction in various wooden properties in North America and California (Su and Scheffrahn, 1990b, Lewis et al., 1996).

Termites of economic importance in the world that caused significant damage to timber-in-service are: 1-*Coptotermes* spp (Asia, Australia, China, Japan, South Africa, Thailand, and USA); 2-*Reticulitermes* species (China, Japan, Southern Europe, and USA); 3-*Mastotermes darwiniensis* (Papua NewGuinea, and Northern Australia); 4-*Hetrotermes* species (Asia, Australia, and Southern USA); 5-*Nasutitermes* species (Australia, and South America); 6-*Psammotermes* and *Anacanthotermes* species (Africa and Middle East); and 7-*Macrotermitina* (Africa and Asia) (Edwards and Mill 1986; Tho, 1992; Pearce, 1997; Ahmed, 2000; Su and Scheffrahn, 2000; Ngee et al., 2004).

1.3. Distribution and numbers of species of termites

Generally, termites are found in the tropical and sub tropical countries and warm temperate areas of the world (Krishna and Weesner, 1969; Edwards and Mill, 1986; Pearce, 1997; Su and Scheffrahn, 2000). The total number of termites in the world was estimated to be at $24x10^{25}$ with density ranging from 230 to 6800 individual /m^2 depending on the type of vegetation (Edwards and Mill, 1986). However, 10 years ago the termite number was estimated to be at 120,000 trillion termites worldwide (Ahmed, 2004).

Termites can be divided into three groups according to nesting habitats and moisture; (1) Subterranean termites (Family: Rhinotermitedae, Mastotermitidae) which dwell the subterranean nests or trees and connect to moisture source through mud tubes, (2) Dampwood termites (Family: Hodotermitidae) which lives in rotten logs or highly moist timber in soil, and (3) Dry wood termites (Family: Kalotermitidae) which nests entirely in timber above ground (Edwards and Mill, 1986; Krishna and Weesner, 1970; Howse, 1970; Pearce, 1997).

There are now over 2700 species of termites described from 282 genera (Kambhampati and Eggleton, 2000; Ngee, 2003). They inhabit approximately 70% of the world, mainly in the tropical and sub-tropical regions extending to some areas in the temperate region (Lee and Chung, 2003). One hundred and eighty three species are known to damage and attack buildings (Su and Scheffrahn, 2000), 70 - 83 of them cause significant damage and the rest are of lesser importance (Edwards and Mill, 1986; Su and Scheffrahn, 2000). In Australia, there are 350 termite species that has been recorded (Ahmed, 2000).

1.4: Subterranean termites

Subterranean termites are considered as one of the most economically important pests in the world (Hickin, 1971; Pearce, 1997; Ahmed, 2000; Su and Scheffrahn, 2000; Perrott, 2003; Ngee, 2003). In addition, they are the most destructive and economically important insect pest of wood and other cellulose products (Beal *et al.*, 1994) and they are responsible for 80% of all termite damage (Su and Scheffrahn, 1990b).

1.5: Damage by subterranean termites

The control and repair of damages caused by subterranean termites in the United States reached a total of US $ 2 billion in the year 2000 (Ngee, 2003). In

California, the subterranean termite and drywood termites are responsible for > 95% of all costs resulting from wood-destroying insects (Lewis and Haverty, 1996). In North America, there are thirty pest species of termites found but two genera of these termite species are most destructive in the United States; *Reticulitermes* and *Coptotermes* (Perrott, 2003; Swoboda, 2004). There are 17 species in Central America and West Indies. Only nine subterranean termite species are considered pest of buildings in North America (Itakura et al., 2006). In Australia, the approximate costs of termite damage exceed US$ 100 million, and $2 million of chemicals are imported annually for termite control (Peters, 1996; Ahmed, 2000). Watson (1990) reported that there were only 20 species of termite of economic importance to timber-in-service in buildings in Australia (Peters, 1996), and *Coptotermes* and *Mastotermes* are the most destructive genera (Peters, 1996; Ahmed, 2000), while Su and Scheffrahn (2000) reported different numbers of termites found in Australia of economic importance species.

In Malaysia, the cost of termite control was estimated to be US$10-12 million in 2003 (Ngee et al., 2004). About 175 - 180 species of termites have been found in Malaysia belonging to a total of 42 genera, a richer fauna than those found in Burma, Thailand, Vietnam, Cambodia, Philippines, Taiwan, Hong Kong, Java, Sumatra and Borneo (Tho, 1992). Less than 10% of them are important pest species (Lee, 2002b). The genus *Coptotermes* is very important to the pest control industry (Sajap et al., 200; Lee, 2002a; 2002b). The numbers of subterranean termite species in India is 26 while 24 species are in tropical Africa. In Japan, *Coptotermes formosanus* (Shiraki) and *Reticulitermes speratus* (Kolbe) are the most important termite pest species (Itakura et al., 2006).

There are several genera of subterranean termites found in the literature. They are *Coptotermes, Odontotermes, Microtermes, Recticulitermes and Hetrotermes* (Su and Scheffrahn, 2000). Eighty percent of subterranean termite is regarded as economically important species worldwide (Su and Scheffrahn, 2000). The genus *Coptotermes* is a worldwide pest termite and has more economic impact than all other termite species founding the world (Edwards and Mill, 1986; Su et al., 1998). All worker termites look for cellulose to feed on and forage in any material such as plants, timbers, papers, books, cartoon, etc. They then bring it to the colony and feed all other nestmates. Therefore, worker termites are the caste that causes all the damages on agriculture crops, buildings and structures. So worker termites are the important target for termite control. The methods for controlling termite which are usetermiticides, graded stones, glass splinters, stainless steel, chemical barriers and baiting systems (Lewis, 1997; Su, 2002; Ngee et al., 2004). These methods are developed in the last few years and require retesting and reevaluation to choose the suitable method and material. One of these methods is termiticides (repellent and non–repellent) were unevaluated, thus this study try to evaluate them with different methods.

1.6 Therefore, the **objectives of this study** are:

1. to determine the lethal concentration (LC_{50}) of some termiticides for controlling the subterranean termite *C. gestroi* .
2. to determine the time of mortality (LT_{50}+LT_{90}) of five termiticides at various concentrations on subterranean termite *C. gestroi* .
3. to determine the feeding performance of subterranean termite *C. gestroi* **.**
4. to investigate the transfer effect of the slow – acting termiticides on subterranean termite *C. gestroi*
5. to determine the penetration of *C. gestroi* in treated - soil in glass tube at various concentrations and thicknesses

CHAPTER 2

LITERATURE REVIEW

2.1: General introduction

Termites are ancients insect, having evolved approximately 200 million years ago (Edwards and Mill, 1986). Studies of fossils petrified termites in the forests of Arizona suggested that termites existed 220 million years ago, which was 100 million years before any other social insects (Pearce, 1997).

The evolution and existence of termites were associated with their nasty problems. Consequently the control of the termites was inevitable and necessary. Unfortunately, there is a lack in the literature that mention when exactly the termite control started. However, Some Chinese literture stated that the early efforts of termites control started around two thousand years ago (Swoboda, 2004).. Certainly, the real chemical control was revealed in 1940s (Aventis, 2003; Perrott, 2003).

2.2: Subterranean termite biology

Termites have a wide range of distribution, throughout different habitats of tropical, subtropical and temperate world regions. Although, subterranean termites live mainly in the tropical forest areas, some studies reported that the termites would be found in an unexpected and extreme habitat such as deserts (Krishna and Weesner, 1970; Edwards and Mill, 1986; Pearce, 1997; Su and Scheffrahn, 2000).

The subterranean termites are social insects with incomplete metamorphosis in their life cycle (Ahmed, 2000). Usually Subterranean termites start with the reproduction through supplementary reproduction, where an existing mature nest starts alate swarming (Edwards and Miller, 1986).

The life cycle starts when the alate adults have been retained by the sexual adults of one caste for a single seasonal migration or dispersal flight (Krishna and Weesner, 1969). The winged forms with long wing-pads are usually present in the colony few months before the flight. The emergence is related to a series of changes in the activity of the colony (Howse, 1970). Soon after reaching maturity, alates (male and female) leave the nest in a swarm and fly up into the air which is called the nuptial flight (Wheeler, 1923). Once the size of colony reached a certain point, the reproduction process launched. However, the time which is needed for reaching that level of size to alert the production process varied between the different species. For example, the colony of *Coptotermes formosanus* may take eight years to reach that threshold size (Pearce, 1997). In addition, before the alate flight, they congregate away from the main colony and then they leave from holes, slited in the ground, mound or wood, or from special flight turrets (Edwards and Mill, 1986).

The number of alates expressed as a percentage of the total colony population. This percentage varies from less than 1% to about 40% according to the species and individual colonies. *Coptotermes lacteus*, for instance, would be expected to produce about 60,000 alates per year (Krishna and Weesner, 1969; Edwards and Mill, 1986).

The pheromones and nutrients influence production of alates (Krishna and Weesner, 1969). The specific time at the day and the year play an important role in the alates emergence. in tropical and warm desert areas the emeregence of alates occurs during the rainy season or seasons soon after the first rains. In warm temperate areas, flights usually occur in the summer, while in every areas, certain species may release their alates at other times during the year (Edwards and Mill, 1986). The time between the appearance of the alates in the nest and date for the first flight is in coincide with the

changes in the weather (Krishna and Weesner, 1969). As Howse (1970) reported, the climatic factors influence the swarming of the termites.

Most of tropical termite species fly in the first part of the rainy season but the actual date differs between species, and determined by the cumulative amount of rain received (Lepage and Darlington, 2000). After the short flight, the alate termites shed their wings. Several stimuli are thought to be involved and in many termites contact between the sexes (male–female) which may trigger the shedding of the wings (Edwards and Mill, 1986).

Pairing or the meeting and association of the sexes generally occur in the substrate after swarming. Therefore, a few types of pairing occur during swarming flight or after being located (Krishna and Weesnar, 1969). Tandem (male follows female very closely) running continues for a few minutes or many hours until a suitable nest site is found. Subterranean termites look for a suitable site to build their nests in the ground (Edwards and Mill, 1986).

Mating occurs after the pair has sealed the entrance and made the first chamber. The female (queen) lays the eggs after few days or (3-6) weeks from the establishment of the royal pair in their first chamber. The number of laid eggs differs among the species. Edwards and Mill (1986) reported that the queen of lower termites lays less than 20 eggs in most species, while higher termites, the queen lays more than 30-100 eggs in the first batch.. *Coptotermes* sp queen produces 100 eggs per day. However, higher number of eggs in a day, 30,000-40,000, could be produced either by *Macrotermes* sp or *Odontotemes obesus* queens (Pearce, 1997).

The incubation period varies among different species. The incubation period for the eggs of *Macrodomes* sp and *Reticulitermes lucifugus* is almost one month.

However, the incubation period of Cryptotermes sp is longer and may range between two to three months (Edwards and Mill, 1986). The king and queen take care of the eggs until they mature. Nymphs undergo several moults and the first batch of eggs hatched develops into workers. The other castes of termites develop from the later egg laying stage (Lee and Chung, 2003).

The size of the first brood of workers, the timing of their appearance and first food collection are important parameters for the success of the young colony. Generally, lower termites have a slow development than higher termites. It takes 67-69 days in *Cubitermes ugandensis* while 27 days in *Macrotermitinae*, *Nasutitermitinae*, and *M.michaelseni*. The first white soldier appeared after 130 days in the field but 50 days in the laboratory (Lepage and Darlington, 2000).

Termite castes can be divided into two major types, the reproductive (queen and king), and the non-reproductive (workers, soldiers and nymphs). The reproductives are divided into primary reproductive (dealate reproductive that formed a new colony after a nuptial flight) and secondary reproductive (insects that differentiated into reproductive in an established colony), and they may be supplementary reproductive (Krishna and Weesner, 1969; Edwards and Mill, 1986; Pearsce, 1997; Ahmed, 2000; 2000; Perrott, 2003). The king and queen will take care of the eggs until they mature.

2.3: The history of the development and the efficacy of termiticides

According to Lee et al. (2003), the insecticides are classified according to their chemical structures, thus dividing them into two main groups; the organic insecticides and the inorganuc insecticides. The organic insecticides are further divided into synthetic insecticides and botanical insecticides.then the organic synthetic

insecticides divided into four class; namely the Chlorinated hydrocarbonsor organochlrones, Organophosphate, Carbamates and Pyrethroids.

Arsenic material or chlorinated hydrocarbons were the earlier material used or reported that have been effective against termites (Roonwal, 1979; Lewis, 1997; Ahmed, 2000; Swoboda, 2004). Meanwhile Aldrin (Shell), chlordane (Velsico), Dieldrin (Shell) and heptachlor (Velsico) were used to control termites for over 25 yeas ago. In addition, Chlorpyrifos (Dursban) and Permethrin (Dragent and Torpedo) have been added to the list (Edwards and Mill, 1986). According to Peters (1996), These insecticides have been used for the past 30 years as termiticides in Australia to control termites. Nevertheless, Creosote, Pentachloro phenol (PCP) and waterborne inorganic chromated copper system were used as wood preservatives treatment (Ahmed, 2000).

In the United States during the 1930-1950, the insecticides which were marketed as soil termiticides are: sodium arsenite, trichloro benzene DDT, penthahloro phenol, creosote, and ethylene dibromide and chlordane, heptachlor, aldrine and dieldrin (Su and Scheffrahn, 2000). In addition, organochlorine insecticides were very effective for the control of termites and other insects in the past and subsequent termiticides which were used after organochlorine and organophosphates insecticides such as Chlorpyrifos, and pyrethroid such as cypermethrin. Both termiticides are effective against subterranean termite (Pearce, 1997; Ngee, 2003). As Pearce (1997) mentioned these termiticide groups were still used in some countries because it is cheaper than the new termiticide groups.

2.3.1: Soil termiticide (Chemical barriers)

Soil termiticides are of the chemical methods to protect building structures from subterranean termite. The termiticides are applied to the soil form the chemical

barrier between the structures and termite (Edwards and Mill, 1986; Lewis, 1997) or to exclude soil borne termites from structures (Su, 1994).there are two groups of termiticides applied into soil, repellent termiticides and non-repellent. The repellent such as chlorpyrifos, while non- repellent as Fipronil, Imidacloprid and Indoxacarb.

2.3.1.1Chlorpyrifos

Chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate] is an organophosphorus insecticide applied into to soil as a termiticide for control termite. It become the only material of proven long-term efficiency available to the pest control industry in the develop countries (Edwards and Mill, 1986). And Chlorpyrifos was less persistent in the environment and widely accepted, although it wasmore toxic to vertebrates than Chlorinated hydrocarbons (Perrott, 2003).

The mode action of Chlorpyrifos is attak the nervous system by binding with acetylcholinesterase (the enzyme which destroys acetylcholine after impulse transmission) and inhibits its function, causing accumulation of acetylcholine at all available receptor sites. This produces repetitive set-off of impulses at the next neural unit (Lee et al., 2003).

Chlopyrifos is a termiticide which provide protection between 1 and 19 years depending on soil type and treatment (Pearce, 1997). Other studies described that chlorpyrifos prevented termite tunneling into treated soil (Su and Scheffrahn, 1990a). Laboratory studies evaluated the effect of chlorpyrifos (Dursban TC) on subterranean termites and it was shown that 1% solution of chlorpyrifos was effective in preventing subterranean termite attack for 21 years (Kard et al., 1989).

The effect of Cholorpyrifos is significant on termite penetration in soil treated at high concentration (500 ppm) where the termites can penetrate the soil only a

few millimeters. Whilst at low concentration (5.0 and 0.5 ppm) termite penetrated the treated soil completely. The effect of Cholorpyrifos on termite mortality was 100% in all treatment thicknesses at 500ppm and 50ppm but at low concentrations (5.0 and 0.5 ppm) the mortality ranged from 45 - 98% across all treated layers (Gaiiliioff and Koeiiler, 2001).

Chlorpyrifos was evaluated as a termiticide against subterranean termite *Coptotermes formosanus* and results showed that chlorpyrifos appeared to have a higher activity than Chloronictinyl Imidacloprid. At the same time, Chlorpyrifos and Imidacloprid had higher activity comparable to the carbamate propoxur (Osbrink et al., 2005). Results of termiticides evaluation in Thailand showed that Chlorpyrifos lasted only 1- 3 years in controlling termites, while Fipronil recorded the longest period (7 years) and Imidacloprid recoded different periods (3 – 6 years) depending on the concentration which plays an important in preventing termite penetration (Vongkaluang et al., 2005).

In another study, *C. formosanus* penetrated through the entire 5cm soil treated with 1ppm chlorpyrifos in a laboratory bioassay (Su and Scheffrahan, 1990a). Meanwhile, the workers of *C. formosanus* penetrated into approximately 4 cm of a 5 cm sand containing 1 ppm chlorpyrifos, and broke through 2.5 cm of sand treated with 1 ppm chlorpyrifos (Su et al., 1995a). However, *C. formosanus* penetrated into approximately 2cm of the a 5cm soil treated with 10 ppm chlorpyrifos and penetrated about 0.63 cm of sand treated with 10 ppm Dursban (Su et al., 1995a).

In addition, chlorpyrifos exhibits its effects even more rapidly than chlorodan. With Chloropyrifos, almost all the termites die from the treatment within the first day, and those which did not die on the first day survived to the end of the

experiments (Su et al., 1987). However, this depends on the concentrations used and the LC_{50} and LC_{90} of the termiticides. For chloropyrifos, it was found that there were significant differences in the tolerance ratios between workers of *C . formosanus* colonies (Osbrink et al., 2001).

According to Osbrink et al. (2001), the range for the LT_{50} was 11.8 to 51.2 min while the LT_{90} was 16.8 to 66.8 min with different colonies of *C. formosanus.* While the results of the LT_{50} for *R. virginicus* ranged from 3 to 11.2 min and the LT_{90} from 22.9 to 64.9 min. However there was no significant difference between the pooled LT_{90} of *C. formosanus* compared with *R. virginicus*. This study was similar to the result of Su and Scheffrahn (1990 a) which described that Chlorpyrifos killed termites quickly upon contact. And they described that the LD_{50} for chlorpyrifos against *C. formosanus* was 3.39µg/g. Also these results are nearly similar to the work of Khoo and Sherman (1979). Hutacharern and Knowles (1974) found that the LD_{50} for chlorpyrifos against *R. flvipes* was 1.74 µg/g. Thus *R. flvipes* was more susceptible to termiticides than *C. formosanus*.

The termite *C. formosanus* tunneled deeper into sand treated with Chlorpyrifos than treated with Pyrethroid termiticides (Su et al., 1993a; 1995a). The other studies determined the minimum concentration of chloropyrifos required to stop termite penetration into treated sand. They were approximatly 50ppm for *C. formosanus* and 10ppm for *R. flavepes* (Su and Scheffrahn, 1990a). However, the efficacy of Chloropyrifos–treated sand at 500 ppm concentration was 14.9 months for *C. formosanus* and 25.4 months for *R. flavipes* (Su et al .,1999).

2.3.1.2: Fipronil

Fipronil is one of the termiticides which used for control termite with low concentration. Fipronil is a 5-amino-1-[2, 6-dichloro-4-(trifluoromethyl) phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile (Hainzl and Casida, 1996). The mode of Fipronil action involves blocking the γ-aminobutyric acid (GABA)-gated chloride channel (Hainzl and Casida, 1996). And as Pearce (1997), described that the mode action of Fipronil is interference with the passage of chloride ions in the nervous system, eventually causing death. According t o Henderson. (2003b), Fipronil and Imidacloprid are more toxic to insects than to mammals because they kill insects through hyperexcitation of the central nervous system.

Termites could penetrate 1.5 cm of soil treated with 100 ppm Fipronil which was equally effective when compared with 0.09 and 0.125% dilution rates. Fipronil controlled 25% of the structures within a month and 77% of structures within two months (Kamble and Davis, 2005).

Osbrink et al. (2001), reported that the LT_{90} of *R. virginicus* had a significantly higher pool than *C.formosanus*. And the results showed that *R. virginicus* when there were less interring colony differences in the response of Fipronil).

Results showed that when Imidacloprid and Fipronil were used in low concentrations, the subterranean termite behavior changed but the periods of efficacy of both termiticide were different. Fipronil treatment was not affecting termites as quickly as Imidacloprid (Henderson, 2003a; 2003b).

2.3.1.3: Imidacloprid

Imidacloprid is *N*-[1-[(6-Chloro-3-pyridyl) methyl]-4, 5-dihydroimidazol-2-yl] nitramide. And it is an insecticide exhibiting low mammalian toxicity. The mode action

of Imidacloprid is on the nervous system of the termites by binds to a postsynapticnicotinic receptor thus blocking neural transmission (Gaiiliioff and Koeiiler, 2001). And when the Imidacloprid action prevents the transmission of the information of the binding sites, resulting a lasting impairment of the nervous system and eventually, death of the insect (Ngee, 2003).

Imidacloprid is a new termiticide which is a slow–acting toxicant even in low concentration. Imidacloprid treatment caused the termites to become sluggish, inhibits grooming and tunneling and eventually caused death (Boucias et al., 1996).

Imidacloprid appears effective against termites. When Imidacloprid used as a treatment under concrete slabs in Arizona, Florida and South Carolina, it remained 100% effective for the first five years of the test. No penetration by termites or damage to wood occurred in the treated plots (Wagner, 2003).

According to Gaiiliioff and Koeiiler (2001), termites could penetrate the sand at least 30% into 10-25 and 50-mm thicknesses treated with 100 ppm Imidacloprid. Generally termites completely penetrated all concentrations less100ppm and thicknesses less 5 mm. Some results showed that when termites were exposed for 4 hours to soil treated with 10ppm Imidacloprid they displayed symptoms of immobiling or impaired mobility. After 4hours of exposure to 100ppm Imidacloprid - treated sand, the termites displayed severe symptoms. After 24 hours of exposure, 96% of these exposed workers were immobile and 4% showed impaired mobility.

Imidacloprid showed latent effect on subterranean termites, *C. formosanus* found near trees treated with 0.1% Imidacloprid adjacent to seven buildings with various distances (1 – 46 m) (Osbrink and Lax, 2003) However, Imidacloprid treated-trees did not control *C. formosanus* populations in independent monitors adjacent to the

treatments. This means that sublethal doses of Imidacloprid can produced an effect on the tunnelling behaviour of the termites (Thorne and Breisch, 2001).

One of the important facters effect on Imidacloprid bioavailability for control termite is the type of soil. Ramakrishna et al (2000) study indicated that organic matter, silt, clay, pH, and cation changes capacity affect on the bioavailability of Imidacloprid effects on termite. The effect of Imidacloprid in reducing termite feeding was the greatest in sand, followed by sandy loam, and silty clay loam.

Sublethal doses of Imidacloprid reduced the grooming behavior of termites permitting the soil borne mychopathogen *Beauveria bassiana* to affect and kill the assayed termite in 7 days (Gaiiliioff and Koeiiler, 2001).

2.3.1.4 Indoxacarb

Indoxacarb, an oxadiazine class of novel chemistry is a newly insecticide with high insecticidal activity and low toxicity (Hu, 2005). Indoxacarb is (S)-methyl 7-chloro-2,5- dihydro-2- [[(methoxycarbonyl) [4-(trifluoromethoxy) phenyl] amino] carbonyl] indeno[1,2-e][1,3,4]oxadiazine-4a(3*H*)-carboxylate.

The mode action of Indoxacarb is occurs via blockage of the sodium channels in the insect nervous system and the mode of entry is via the stomach and contact routes (Hu, 2005).

Indoxacarb causes mortality in subterranean termite *Coptotermes formosanus* and eastern subterranean termite *Reticulitermes flavipes*. Hu (2005) found that Indoxacarb showed higher mortality than controls at all treatment thicknesses ≥10 ppm. Concentration and thickness of treated soil with Indoxacarb affected significantly termite mortality (Hu, 2005). Eastern subterranean termite *R. flavipes* was more susceptible to Indoxacarb than *C. formosanus*. *R. flavipes* and *C. formosanus* and

completely penetrated through all treatment thickness of Indoxacarb–treated soil at all concentrations (Hu, 2005).

2.3.2: The other methods for termite control

Due to the long residual activity for some termiticides, or the impact of active material of termiticides on the soil vertebrate, some researchers investigated new methods that avoid using chemicals. Other methods of control are physical barriers and biological control.

2.3.3.1: Physical barriers

Some studies were carried out to investigate the use of sand particles as physical barriers to prevent subterranean termite from penetrating structures (Su and Scheffrahn, 1992; Ebeling and Pence, 1957; Tamashiro et al., 1991; Mauldin and Kard, 1996; Lewis and Haverty, 1996). Other materials that has been used as physical barriers were graded stones, glass splinters and stainless steel (Lewis, 1997; Peters, 1996; Ngee, 2003; Grace et al., 1996b; Cornelius, 2005)

2.3.2.2: Biological control

The biological control of termite includes nematodes, fungi and viruses (Pearce, 1997).

2.3.4.1: Nematode

Poinar et al. (1989) reported that high mortality (≥ 80%) of *Reticulitermes spp* have been generated under laboratory conditions when high concentration of nematodes were applied (Yu ,et al 2006). Some studies which used nematodes such as *Steinernema carpocapsae* (Breton), *S. riobrave* Cabanillas, Poinar and Raulston (TX), *Heterorhabditis indica* Poinr, Kanunakar and David and *H. bacteriophora* Poinar (HP88) against subterranean termites *Coptotermes formosanus* and *R. favipes* showed

that all the nematodes were effective against *C. formosanus* in a petri dish test at a concentration of 400 nematodes *S. riobrave* per termite,. They had not detected any effect against *R. flavipes* even at a rate of 2,000 nematodes per termite (Wang et al., 2002).

According to Pearce (1997) and Epsky and Capinera (1988), who examined the effect of the nematode *Steinernema* against *C.formosanus* and *Rticulitermes speratus* at a rate of 4000 -8000 nematodes in 3 ml, and the results showed that these nematode were effective on the termites. Fujii, (1975) indicated that Weiser was evaluated the nematode *Nemoaplectana carpocapsae* against formosanus subterranean termite as the first nematode used against termite. While, Mauldin and Beal, (1989) reported that the nematode *Steinernema feltiae* was used against eastern subterranean termite *R. flavipes* to test the potential use as entomogenous nematode, but the results were not very effective .

2.3.4.2: Fungi

Metarhizium anisopliae (Metschnikoff) and *Beauveria bassiana* pathogenic fungi appeared to be more successful for termite control (Pearce, 1997). Some studies indicated the potential use of the pathogenic *M. anisopliae* (Metschnikoff) for the control of *N. exitiosus* (Hanel and Watson, 1983). While in China, Dong et al. (2006) found that the efficacy of a new virulent *M. anisopliae*. It was highly infectious and virulent against termite *Odontotermes formosanus*. It caused almost100% mortality to the termites after 3 days post inoculation with concentration of $3x10^8$ conidia / ml. In other studies, the efficacy of a new virulent *M. anisopliae* strain (SRRC 2558) was evaluated against the eastern subterranean termite, *R. falvipes* and the formosanus

subterranean termite *C. formosanus* and highly infectious against the termites. It caused 100% mortality to groups of 100 *R. flavipes* workers at a concentration of $\geq$ 3x 10^3 conidia / cm^3 (Wang and Powell, 2004).

2.3.5: The baiting system

The baiting system is a new method to control subterranean termites. The baiting system utilizes the advantage of social nature and foraging behavior of subterranean termites where food sharing among the workers and nestmates via trophallaxis could enable the transfer of slow –acting toxicant to the whole colony (Lee and Chung, 2003).

Baiting technique is one of the widely accepted methods for controlling subterranean termite as a long lasting and cost – effective method (Sajap et al., 2005; Kubota et al., 2006; Getty et al., 2005). Baiting system is used to control subterranean termite near the structure (Su, 2002). It can operable to eliminatinl an entire colony of subterranean termites (Esenther and Gray,1968; Su et al., 1982; Su, 1994a; Su et al., 1995b; Su and Scheffrahn, 1996a and b; Su et al., 1997; Su and Hsu, 2003; Sajap et al., 2000; Sajap et al., 2005; Chambers and Benson, 1995; Grace et al., 1996a; DeMark et al., 1995; Peters and Fitzgerald; 1998 Prabhakaran, 2001; Thorne and Forschler, 2000; Grace and Su, 2001; Klein, 2002; Lee, 2002a and b).

Termite bait acts by eliminating or suppressing colony that infest the structures (Ngee et al., 2004) and the elimination occurs because the slow acting nature of the ingested toxicant allows foraging termites to return to the colony and transfer the toxic material to the unexposed nestmates before killing the carrier (Sheets et al., 2000). Slow–acting and non-repellent active ingredients in termite baiting is very important for successful control (Su et al., 1982).

Metabolic inhibitors like hydramethylnon which is used in baits to control *Coptotermes formosanus* in the laboratory and field has shown that it can eliminate colonies by using 0.3% and allows the transfer of lethal dose within the termite population (Su et al 1982; Klein, 2002). Another study found that the LD_{50} of hydramethylnon was different for two subterranean termite species; 56.09µg/g for *C. formosanus* and31.25µg/g for *R. flavipes* (Su et al., 1994 b).

Another metabolic inhibitor used in baiting system is Sulfluramid which can affect subterranean termite *C. formosanus* in low concentrations (100 ppm) (Grace et al., 2000). The LD_{50} of Sulfluramid was found to be different for *C. formosanus* and *R. flavipes,* 6.95µg /g and 60.6µg /g, respectively (Su and Scheffrahn, 1991).

Other results showed that LD_{50} of Sulfluramid was 4.31µg/g and 41.34µg/g for *C. formosanus* and *R. flavipes* respectively (Su et al., 1994 b). There are some other materials used under this group such as Borate (Jones, 1991), boric acid (Mori, 1987) Diiodomethhyl para-toly sulfone (Su and Scheffrahn, 1988) and Mirex (Su and Scheffrahn, 1991). All these materials showed changes to foraging behavior of termites (Su et al., 1994; Ngee, 2003).

Another metabolic inhibitor is Hexaflurmuron which is used in baiting systems that do not require the use of additional pesticide, while Sulflurmuron is recommended for use in conjunction with termiticides (Su, 2002). The total amount of hexaflumuron needed to eliminate three colonies of *C. gestroi* were different; 924, 1221, 1456 mg for each colony (Lee, 2002a). Meanwhile for *Coptotermes havilandi*, a total of 0.89-1.47g of hexaflumdron was needed to eliminate the colony (Lee, 2002b).

Getty et al (2005) reported that a colony of *Reticulitermes spp* was baited with 0.5% hexaflumuron. Sixty days later, the termite *Reticulitermes spp* was absent from all monitoring Sentricon stations. Meanwhile other field evaluation studies (Su, 1994a) showed approximately 4-1,500mg of hexaflumuron was needed for 90-100% elimination of subterranean termite populations and concluded that 20.3g of hexaflumuron eliminated 730,000 termites in two months (Su,1994a).

Results obtained from the field suggested that continuous baiting program, with Sentricon® system, had a significant impact on the subterranean termite population (Getty et al., 2005). Hexaflumuron and noviflumuron were evaluated for effectiveness against subterranean termites in Malaysia and the results showed that the hexaflumuron baits could effectively eliminate *C. gestroi* and *C. curvignathus*, while noviflumuron was used as above ground baiting for control *C. gestroi*. The colonies of *C. gestroi* succumbed to the effects of the toxicant of 0.5% noviflumuron (Sajap et al., 2005).

The time required for eliminating colonies of *C. havilandi* was 3-5 months when hexaflumuron was used as a bait (Su et al., 2000). However, when it is used to control or eliminate *C. curvignathus*, it took 25-44 days (Sajap et al., 2000). The difference in time for the elimination of termites may be due to termite species (Su et al., 2001) or different repress toxicity for different termite species. Another chitin synthesis inhibitor, Diflubenzuron, was effective against eastern subterranean termite *R. falvipes* (Su and Scheffrahn, 1993b).

The biological activity of Lufenuron resembled that of Diflubenzuron and was less effective against *C. formosanus* than against *R. flavipes* (Su and Scheffrahn,

1996a). Some studies evaluated the effectiveness and the efficiency of baiting system by using Insect Growth Regulator (I G R) in termite baiting (Cornelius, 2005; Peters and Broadbent, 2005; Kubota et al., 2006; Su and Scheffrahn,1989; Su and Scheffrahn,1990b; Su and Scheffrahn,1996a; Su et al.,1991; Su et al.,1995b; Su et al.,1997).

IGRs used in baiting system are slow acting chemicals to reduce termite population. So, the effect of IGRs on termite colonies causes a high proportion of pseudo–gates, worker nymphs or larvae to moult into pre-solider or non functional inter castes (Edwards and Mill, 1989; Lee and Chung, 2003; Su and Scheffrahn, 1989).

According to Perrott (2003), many slow-acting toxicants have been impregnated into wooden bait blocks and tested against subterranean termite such as Mirex, Hydramethylnon, Avermectin B, A-9248 (diiodmethyl para-toyl sulfone), Sulfluramid, Hexaflumuron and diflubenzuron. Some studies indicated that IGRs responded better against termite species which has lower natural soldiers such as *K. flavicollis* and *Reticulitermes* species (1-2% soldier) than *C. amanii* and *C . formosanus* (10-15% soldier which has a higher number of soldiers. (Su and Scheffrahn, 1993b). IGRs are promising candidates for bait control for termites because of their gradual and cumulative mode of action (Su and Scheffrahn, 1993b).

CHAPTER 3

LABORATORY EVALUATION OF SOME TERMITICIDES AGAINST SUBTERRANEAN TERMITE *Coptotermes gestroi*

3.1 INTRODUCTION

The cost of termite control in Malaysia was estimated to be US$ 10-12 million (Lee, 2002b; Ngee et al., 2004). Generally, around 90% of the infestation on structure and buildings were caused by several species of *Coptotermes* (Lee, 2002a). Subterranean termite *C. gestroi* is considered as a pest species in South East Asia and Brazil and a huge damage has been caused by such species in these regions (Kirton and Brown, 2003; Costa-Leonardo et al., 2004). In fact *C. gestroi* is the most aggressive species that causes 63 - 90% of all damages in urban structures and buildings in Malaysia, Thailand and Singapore (Sornnuwat et al.,1996a, b and c; Lee, 2002a; Lee et al., 2003; Lee et al., 2007). Doors, window frames, and parquet floors were found to be the most prone to termite attack within the structures (Lee, 2002).

Laboratory studies indicated that some termiticides such as Durspan TC4 and Cypermethrin have the ability to control termite successfully by oral or feeding tests. Both termiticides were shown to be effective against subterranean termites. Meanwhile, new non-repellent termiticides such as Premise® 200 SC contain Imidaclprid while Regent and Agenda contain Fipronil have shown a high efficacy on termite control. They are slow-acting toxicants, safe to use and environmentally acceptable (Pearce, 1997; Osbrink et al., 2001; Ngee, 2003; Osbrink et al., 2005).

For the purpose of testing termiticides, laboratory based studies usually used the force-feeding test, choice feeding test or topical toxicity test, to determine LD_{50} and lethal time (LT_{50} and LT_{90}) (Su and Scheffrahn, 1988; Su et al., 1994).