พฤติกรรมการหาอาหารและการเลือกชนิดอาหารของมดละเอียดท้องดำ *Trichomyrmex destructor* (Jerdon, 1851)

นายอัศวิทย์ ศรีศักราภิคุปต์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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FORAGING BEHAVIOR AND FOOD PREFERENCE OF SINGAPORE ANT *Trichomyrmex destructor* (Jerdon, 1851)

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A Thesis Submitted in Partial Fulfillment of the Requirements
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อัศวิทย์ ศรีศักราภิคุปต์ : พฤติกรรมการหาอาหารและการเลือกชนิดอาหารของมดละเอียดท้องดำ*Trichomyrmex destructor* (Jerdon, 1851) (FORAGING BEHAVIOR AND FOOD PREFERENCE OF SINGAPORE ANT *Trichomyrmex destructor* (Jerdon, 1851)) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. ดวงแข สิทธิเจริญชัย, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร. อาจอง ประทัตสุนทรสาร, หน้า.

การศึกษาพฤติกรรมการหาอาหารและเลือกอาหารของมดละเอียดท้องดำ *Trichomyrmex destructor* ตั้งแต่เดือนธันวาคม 2557 ถึง พฤศจิกายน 2558 พบว่ากิจกรรมการหาอาหารของมดเกิดขึ้นสูงสุดช่วงเวลา16.00-18.00 น. และช่วงเวลาพระอาทิตย์ขึ้น-ตกไม่มีผลต่อเวลาการเริ่มหรือหยุดออกหาอาหารของมด ถึงแม้ว่ามดละเอียดท้องดำออกหาอาหารทั้งกลางวันและกลางคืนแต่มีกิจกรรมการหาอาหารในช่วงกลางวันมากกว่าช่วงกลางคืน ค่าเฉลี่ยจำนวนมดที่กลับรังในกิจกรรมการหาอาหารในช่วงที่มีเหยื่อล่อ (75.41 ± 3.18 ตัว/นาที) สูงกว่ากิจกรรมการหาอาหารในช่วงที่ไม่มีเหยื่อล่อ (31.44 ± 1.53 ตัว/นาที) อย่างมีนัยสำคัญทางสถิติ และค่าเฉลี่ยจำนวนมดที่มีกิจกรรมการหาอาหารในช่วงฤดูฝน (56.38 ± 2.37 ตัว/นาที) สูงกว่าในช่วงฤดูแล้ง (47.18 ± 3.22 ตัว/นาที) ทั้งที่มีและไม่มีเหยื่อล่อ อุณหภูมิพื้นผิวและอุณหภูมิอากาศมีอิทธิพลต่อกิจกรรมการหาอาหารของมดละเอียดท้องดำอย่างมีนัยสำคัญทางสถิติ ขณะที่ความชื้นสัมพัทธ์ไม่มีผลต่อกิจกรรมการหาอาหารของมดอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ในช่วงที่มีเหยื่อล่อ มดละเอียดท้องดำจะมีการออกหาอาหารในช่วงปัจจัยทางกายภาพที่กว้างกว่าในช่วงที่ไม่มีเหยื่อล่อ ค่าเฉลี่ยระยะทางออกหาอาหารของมดละเอียดท้องดำในช่วงฤดูแล้ง (1.33 ± 0.16 เมตร) ไกลกว่าค่าเฉลี่ยระยะทางออกหาอาหารในช่วงฤดูฝน (0.76 ± 0.05 เมตร) อย่างมีนัยสำคัญทางสถิติ ในรอบปีมดละเอียดท้องดำเลือกเหยื่อทั้งที่เป็นคาร์โบไฮเดรตและโปรตีน โดยเลือกแตกต่างกันระหว่างฤดู ในฤดูแล้ง เหยื่อประเภทโปรตีนถูกเลือกมากที่สุด ขณะในฤดูฝน เหยื่อประเภทคาร์โบไฮเดรตถูกเลือกมากที่สุด นอกจากนี้ค่าเฉลี่ยจำนวนมดละเอียดท้องดำที่เลือกเหยื่อที่มีสภาพเป็นของแข็ง (34.73 ± 1.75 ตัว/กับดัก) ยังมากกว่าเหยื่อที่มีสภาพไม่เป็นของแข็ง(14.41 ± 1.00 ตัว/กับดัก) อย่างมีนัยสำคัญทางสถิติ การศึกษาความชุกของมดละเอียดท้องดำในภาคสนามพบว่าความชุกของมดละเอียดท้องดำมีความแปรผันตลอดทั้งปีและพบว่าความชุกของมดละเอียดท้องดำมีความชุกน้อยกว่าช่วงอื่นของปีในช่วงฤดูแล้ง

ภาควิชา	ชีววิทยา	ลายมือชื่อนิสิต ....
สาขาวิชา	สัตววิทยา	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
ปีการศึกษา	2559	ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

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USSAWIT SRISAKRAPIKOOP: FORAGING BEHAVIOR AND FOOD PREFERENCE OF SINGAPORE ANT *Trichomyrmex destructor* (Jerdon, 1851). ADVISOR: ASST. PROF. DUANGKHAE SITTHICHAROENCHAI, Ph.D., CO-ADVISOR: ASST. PROF. ART-ONG PRADATSUNDARASAR, Ph.D., pp.

Foraging behavior and food preference of *Trichomyrmex destructor* were conducted during December 2014 – November 2015. The foraging activity of the ant peaked at 4 pm to 6 pm. Neither emergent time nor cessation time was influenced by sunrise-sunset times. Although Singapore ant tended to forage throughout the day, the ant activity during daytime was higher than the nighttime. The mean number of returning ants in foraging activity with bait (75.41 ± 3.18 ants/minute) was significantly higher than the regular foraging activity (31.44 ± 1.53 ants/minute). The mean number of ant foraging activity in the wet season (56.38 ± 2.37 ants/minute) was greater than the dry season (47.18 ± 3.22 ants/minute) in both conditions. Surface temperature and air temperature significantly influenced the ant foraging activity, while relative humidity was not significant. Species response curve results revealed that Singapore ants foraged in the wider physical ranges when bait was presented. The foraging distance of Singapore ant in the dry season (1.33 ± 0.16 m) was significantly greater than the wet season (0.76 ± 0.05 m). The ant generally preferred both carbohydrate and protein based baits throughout the year with seasonal difference in preference. During the dry season, protein baits were most preferred by ants, while carbohydrate baits were more preferred during the wet season. The mean number of ants trapped in the solid baits (34.73 ± 1.75 ants/trap) was significantly greater than the liquefied baits (14.41 ± 1.00 ants/trap). The ant abundance in the field condition showed the fluctuating pattern across the year but the ant abundance was slightly low during the dry season.

Department:	Biology	Student's Signature	
Field of Study:	Zoology	Advisor's Signature	
Academic Year:	2016	Co-Advisor's Signature	

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CHAPTER I

INTRODUCTION

Ants are well known as one of the major pests in human daily life especially household pests. Ants can be found both in the natural environment and urban environment. The presence of certain ants in urban environment leads ants close and cause nuisance to human. This closeness to human also leads to the household and economic damages. According to the household pest questionnaires conducted in Malaysia during 1983–2001, the results showed that ants were ranked the third major household pest behind mosquitoes and cockroaches, respectively (Lee, 2002). These evidences emphasize ants as one of the crucial and problematic pests in Southeast Asia.

Singapore ant or *Trichomyrmex destructor* (which previously well known as *Monomorium destructor*) (Ward et al., 2015) is classified as an insect in Order Hymenoptera, Family Formicidae and Subfamily Myrmicinae. It may originate from India and it is widely distributed from the tropical zone to the temperate zone by commercial transportation (Bolton, 1987). Thus, this ant is commonly found throughout Thailand (Jaitrong and Nabhitabhata, 2005). Due to the fact that Singapore ant has a good capability of adaptation to the new environment, so this ant can be found both in the natural environment and in the urban environment.

Different ant species show different foraging times (Bernstein, 1979). There are many factors which influence ant foraging activity including both biotic and abiotic factors. Moreover, some ants can alter their peak activities to match the fluctuating environments (Holldobler and Wilson, 1990). All of these, ambient temperature, soil surface temperature, light and humidity, are the examples of abiotic factors affecting ant foraging activity (Bernstein, 1979; Vowles, 1995). At the particular temperature and the presence or absence of light can impose the initiation or cessation of foraging time while soil surface temperature correlates with foraging activity of many ant species (Bernstein, 1979; Narendra et al., 2010; Schneirla, 1938). Humidity also plays an important role on ant foraging activity. A lot of researches pointed out that humidity with temperature as well were the factors that limited ant foraging activity (Abril et al., 2007; Mashaly et al., 2013; Raimundo et al., 2009). For instance, *Eciton* ants increased their activities during the morning and declined during the midday to reduce the loss of water when they reached the lowest humidity and the highest temperature (Vowles, 1995).

Furthermore, biotic factors also influence ant foraging activity. For example, the returning ant especially the one with food can boost foraging activity (Carroll and Janzen, 1973; Gordon et al., 2013). The presence of parasitoid flies in the genus *Pseudacteon* against Argentine ant, *Linepithema humile*, also cause this kind of ants to abandon their food resources (Orr and Seike, 1998). Thus, ant foraging activity is a result of the unique morphological, physiological and behavioral characteristics of the ant foragers (Bernstein, 1979). The presence of particular ant species can affect other ant species as well. For example, *Lasius pallitarsis* significantly decreases their foraging activity when physically contact with the deadly competitors *Formica subnuda* (Nonacs and Dill, 1988).

There are many factors which influence the ant territory size such as food availability, defensive cost and life history (Gordon, 1995). Foraging range is one of the interesting aspects in ant foraging behavior. Foraging range depends on the age of the red harvester ant, *Pogonomyrmex barbatus*, colony and also depends on the colony location of conspecific ant in Florida harvester ant, *Pogonomyrmex badius* (Gordon, 1995; Harrison and Gentry, 1981). The individual *Cataglyphis bicolor*, the desert ant, exhibited the different foraging approaches caused by an intrinsic factor. The factor was “training bias”, the first few successful excursions, which was the factor determining the further foraging manner (Schmid-Hempel, 1984). Moreover, at the first round of each foraging, *C. bicolor* individually searched in the random direction (Harkness and Maroudas, 1985). In this study, the individual ant (not the one in trail) which engaged in searching food was used as a model to study foraging range.

Food preference was also affected by the different seasons in red imported fire ant, *Solenopsis invicta* (Cook et al., 2011; Stein et al., 1990). Moreover, the research about bait formulations was developed for ant management and control. Most indoor pest ants preferred bait formulation made of high liquid content which relatively involved with abiotic factors (Lee, 2008). The size of granular bait is one of the important issues which promote the success of ant control by baiting. The former study substantiated that the most preferable particle size of each ant species is correlated with the worker head width (Hooper et al., 2002). The difference of microhabitats between arboreal and terrestrial ants also determined the food preference in ants under the different seasons (Hahn and Wheeler, 2002).

Ant larvae influence both colony preference and regulation of nutrient intake. Due to the fact that worker ants cannot process solid food, so the ant larvae have a crucial task to liquidize the food for worker ants (Cassill et al., 2005). The study in Argentine ant, *Linepithema humile,* demonstrated that protein food was mainly consumed by queens and larvae, while carbohydrate food was mainly eaten by workers (Markin, 1970). Another study on *L. humile* showed the similar trends that the worker ants preferred carbohydrate food during pupae were emerging and the worker ants need protein food when queens laid eggs and larvae were developing (Abril et al., 2007). In addition, when larvae are present, *Myrmecia* workers shifted their food preference and *Myrmica rubra* workers augmented protein food proportion (Vowles, 1995).

Many mentioned studies above reported that different seasons have influence on ant foraging activity according to the different ambient temperature and humidity. This seasonal change also leads to the different of ant food preferences which can be observed in different periods of the year both under laboratory condition and field condition (Cook et al., 2011; Stein et al., 1990), and different bait formulations also have influence on different ant species' food preference (Lee, 2008).

Thus, these are interesting to conduct experiments to prove whether the different seasons in urban environment have impacts on foraging activity and food preference of Singapore ant as well as the impacts of bait formulation on ant preference. However, most studies that relate to pest ants usually aim to commercially studies such as the study of the formulations of the ant baits (Eow and Lee, 2007; Lee, 2008). In addition, many experiments about pest ants were usually conducted in laboratory condition which no change in environmental factors. In contrast, the ecological studies of pest ants are still limited, therefore, this research would fulfill the biological knowledge about foraging behavior of this ant under ecological aspect in field condition covering all seasons in Thailand and would be applied to use in pest control and management program in the future.

The overall objectives of this study are to examine the foraging activity and feeding preference of the Singapore ant. The specific aims were:

1. To study whether Singapore ants alter their foraging activity and foraging range according to the change in seasons.

2. To study whether Singapore ants switch their food preference due to seasonal change.
3. To investigate the change of Singapore ant abundance all year round.

CHAPTER II

LITERATURE REVIEW

2.1 Classification and general description

Ants are classified as an insect in Order Hymenoptera, Family Formicidae which consists of 21 subfamilies. They have the highest number of species of approximately 15,000 compared to all other social insects, about 9,000-10,000 species have been described (Bolton, 1994; Lach et al., 2010).

Trichomyrmex destructor has been previously well known for a long time as *Monomorium destructor* until recently there was a published article related to phylogeny of ants in subfamily Myrmicinae. This article proved that the *'Monomorium'* *scabriceps*– and *destructor*– groups formed their own clade outside the rest of *Monomorium* with high statistical support, so the species in *scabriceps* – and *destructor* – groups were moved to the new resurrected genus named *Trichomyrmex* (Ward et al., 2015).

T. destructor or Singapore ant was first described by Jerdon in 1851. He described that this ant was "common in all parts of India", so many research articles concluded that this ant species may originate from Asia. Singapore ant has widely distributed especially from tropical zone to temperate zone (Figure 1) via commercial transportation (Bolton, 1987; Wetterer, 2009).

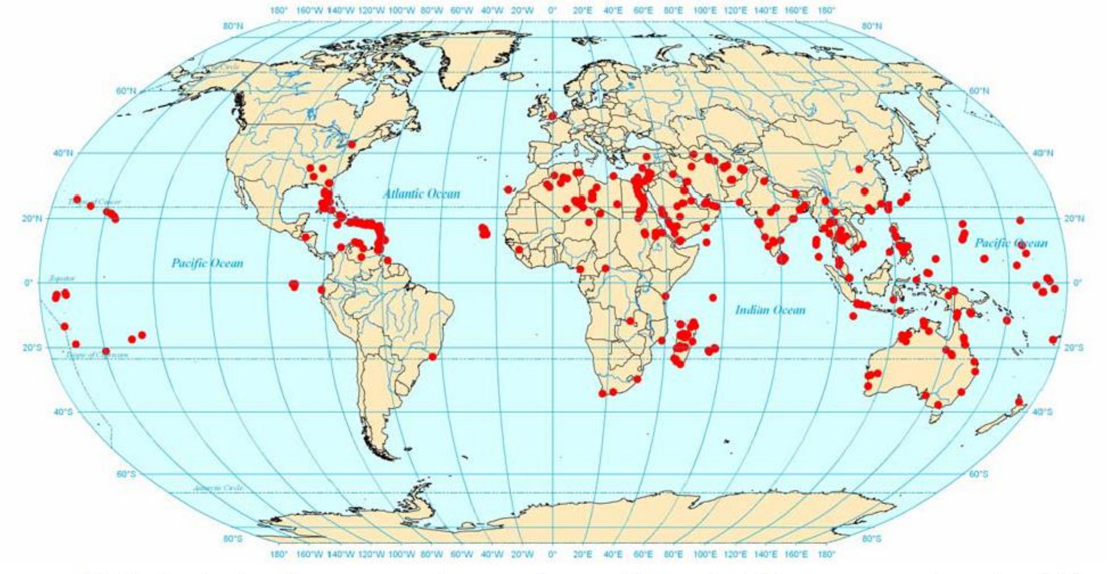

Figure 1 Worldwide distribution of *Trichomyrmex destructor* (Wetterer, 2009)

T. destructor is classified in subfamily Myrmicinae which is the largest ant subfamily with more than 6,700 described species. This subfamily contains a wide range of foraging traits such as omnivores, predators, scavengers, seed harvesters, primitive fungus-growers and leaf-cutting ants (Lach et al., 2010).

Workers of Singapore ant show variation in sizes range from 1.8–3.5 millimeters (Figure 2). The worker’s head to post-petiole is glossy yellow which varies in shade from light yellow to dull brownish yellow while its gaster is always dark brown to nearly black (Bolton, 1987; Wetterer, 2009) (Figure 3). The outstanding character of *T. destructor* is the porous surface (sculptured) which is found only on mesonotum and propodeum, while pronotum is smooth and shiny. In addition, the propodeum of Singapore ant also lacks spines or teeth (Smith, 1965) (Figure 4-5).

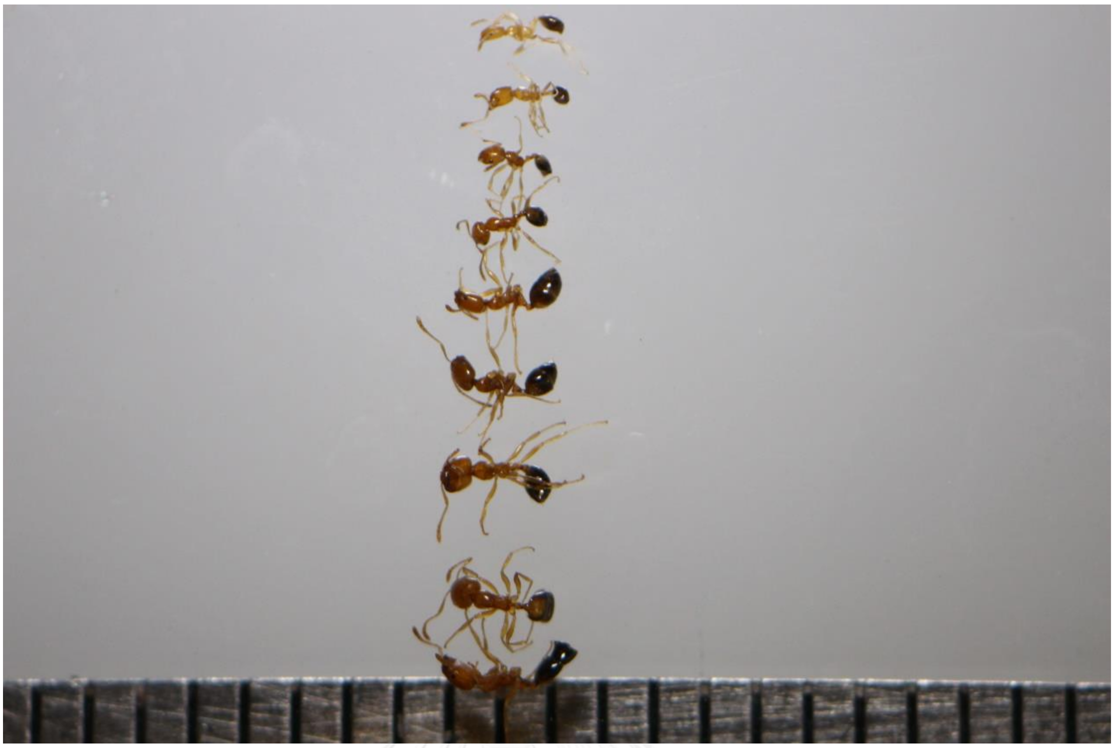

Figure 2 The variation in sizes of *Trichomyrmex destructor* workers

Figure 3 *Trichomyrmex destructor* worker

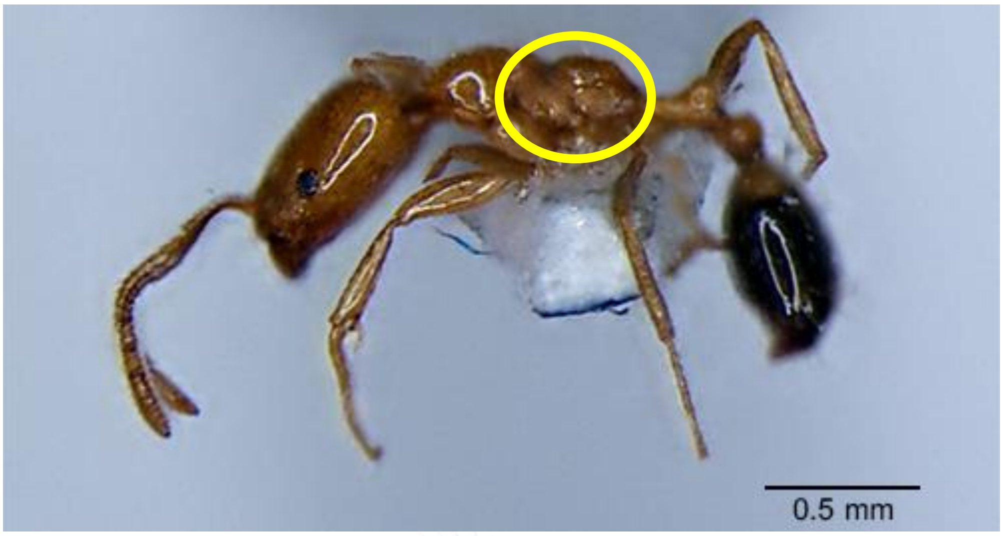

Figure 4 The porous surface (in the circle) which is present only on mesonotum and propodeum of *Trichomyrmex destructor* worker.

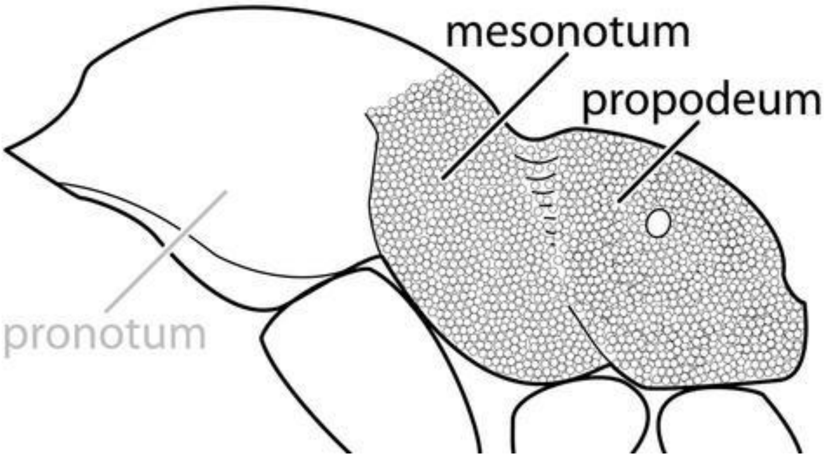

Figure 5 The sketch of porous surface of *Trichomyrmex destructor* (from http://antkey.org/taxonomy/term/4861/media)

2.2 Biology of *Trichomyrmex destructor*

Singapore ant is one of the common pests in the urban environment which nests both indoors and outdoors (Smith, 1965). It nests in soil, natural crevices and building crevices; and this omnivorous ant consumes broad range of household food or any food available including dead insects, insect eggs, honeydew from hemipteran, nectar and seeds (Harris, (n.d.); Smith, 1965). Singapore ants form large polygynous colonies which there are many queens in a single colony and they are polydomous (multiple nests) which builds spatially separated nests but socially connected. Moreover, their social structure is unicoloniality; a colony structure that is a consequence of extreme polygyny and polydomy, and no distinct boundaries among nests that allow population to freely move along the nests (Debout et al., 2007; Halantera et al., 2009; Lach et al., 2010). Unlike most ant species that found new colony by nuptial flight method, Singapore ants found a new colony by colony budding method which a queen or queens leave the natal nest on foot with workers to found a new colony. If the new colony is adjacent to the natal nest and remains contact to each other, polydomy arises. Although all workers are female, they cannot reproduce anymore because they are sterile (Halantera et al., 2009).

2.3 Foraging behavior

Singapore ant is a slow-moving ant that usually forms trails to the food resources by using trail pheromone. Furthermore, foraging strategy of this ant is “mass recruitment” of which a large number of ants are rapidly attracted by trail pheromone left by the successful foragers (Lach et al., 2010).

2.3.1 Foraging activity

Different ant species exhibit different durations of foraging such as in two sympatric *Myrmecia* ants which *M. croslandi* is diurnal while *M. pyriformis* is nocturnal (Jayatilaka et al., 2011). However, some ants can shift their peak activities back and forth to conform to environmental conditions as in leafcutter ant *Atta cephalotes* (Holldobler and Wilson, 1990). Although the data of foraging activity of Singapore ant is quite limited, there still are several studies about foraging activity of pest ants.

In other crucial pest ants, *Monomorium pharaonis* (known as Pharaoh ant) and *Monomorium orientale*, the former is most active during evening and the latter is most active during early morning (its peak activity is between 00:00-04:00 AM) (Loke and Lee, 2005; Mallis, 1997). This shows that ants in the same genus can exhibit the difference in foraging hours.

Moreover, the physical factors such as light, temperature and humidity also have influence on ant foraging activity (Vowles, 1995). For example, light is a determining factor of emergent time of subterranean *Eciton* ants. Their activities increase in the morning, decrease at noon, boost again in the afternoon and decline again when the light fades. In some desert ants, their activities increase especially in the presence of moonlight (Vowles, 1995). Furthermore, the study of foraging activity of a pest ant, *Tapinoma indicum* (known as ghost ant), in Malaysia showed that there were more ants foraging at night because of the lower temperature and higher humidity. So air temperature has a negative influence on ghost ant's foraging activity whereas air humidity has a positive influence with its morning peak activity at 07:30 AM (Chong and Lee, 2006).

Likewise, the study of foraging activity of Argentine ant, *Linepithema humile*, at cork oak secondary forest in northeast Iberian Peninsula, Spain showed that the ant activity sharply decreased in winter while its activity in summer and spring was limited by low humidity and high temperature. During summer and spring, Argentine ant activity was continual, but was greater at night. These can be explained that the abiotic factor (high temperature with low humidity) would influence on the ant activity, while in winter when the temperature dropped below 5°C, Argentine ant completely ceased all foraging activity (Abril et al., 2007).

Raimundo et al. (2009) studied the foraging behavior of ground dwelling ant *Odontomachus chelifer* (Latreille) in reserved forest in Southeastern Brazil. The result showed that the foraging activity in wet-warm season (November-March) was greater than in dry-cold season (April-October). This trend can be explained that, in wet-warm period, there was more litter dwelling prey. However, the *O. chelifer* foraged only the nocturnal time to avoid interspecific competition with diurnal *Pachycondyla striata* ant which also foraged prey on the litter.

Mashaly et al. (2013) studied the foraging activity of *Pachycondyla sennaarensis* (Mayr) in the field over two years throughout all four seasons. There were significant differences in foraging activity among different seasons. The foraging activity declined in autumn and winter. In contrast, the foraging activity was greater in spring and summer which in the summer, the activity was more eminent during the cooler period. The researchers suggested that the activity of *P. sennaarensis* was affected by time, ambient temperature and relative humidity.

Besides the abiotic factors, biotic factors also influence ant foraging activity. Gordon et al. (2013) studied the foraging activity of harvester ant, *Pogonomyrmex barbatus*, at New Mexico, USA. They found that there was an effect of returning foragers on the rate of outgoing foragers which depended on humidity. This can be explained that humidity may increase the effectiveness of chemical cues in interaction between outgoing and returning foragers.

2.3.2 Food preference

Most ants are accounted as omnivorous which include predation, scavenging and consumption of plant-based resources. However, ants are holometabolous (complete metamorphosis) insects which require different kinds of nutrition for each stage of their life cycle. Larvae require the diet for growth while the workers need food for maintenance of their body functions (Lach et al., 2010). For instance, the study of food distribution in *Linepithema humile* (formerly *Iridomyrmex humilis*) (Shattuck, 1992) or known as Argentine ant in laboratory, the carbohydrate was mainly utilized by workers while considerable amount of protein was consumed by queens and larvae (Markin, 1970).

Larvae also have influences on worker's food preference. Normally, *Myrmecia* workers are nectivorous but, when the larvae are present, the workers shift their foraging behavior by bringing back insect booties for the larvae. Likewise, *Myrmica rubra* increase both their proportion of protein food and their foraging activity when the larvae are present (Vowles, 1995). Because larval development is a stage of growth and increases its individual biomass, protein is so important for this stage.

Ants have their own food preference regulation based on macronutrients. Cook et al. (2010) conducted an experiment by giving food in granule forms which varied in carbohydrate-protein ratio to red imported fire ant, *Solenopsis invicta*. They found that food collection was affected by carbohydrate-protein ratio. The treatments which received equal ratio of carbohydrate and protein and protein-biased foods were collected the most amount of food. This can be explained that the collection of large amount of equal ratio and protein based foods was a result from the ants tried to meet their carbohydrate requirement when the carbohydrate food was scarce.

Season is another factor that influences food preference in some ants such as red imported fire ant, *Solenopsis invicta*. This study was conducted in summer and fall by collecting red imported fire ant colonies from a field at the Riverside campus of Texas A&M University, USA. Then, ants from summer and fall were fed with various protein-carbohydrate ratios under laboratory condition. The results showed that the ants collected in summer gathered more food than the ants collected in fall, but the ants from these two seasons showed similar regulation of protein consumption. This can be explained by the intake of too much protein which is toxic to both workers and larvae. Thus, this study showed the different proportions in food selection (protein and carbohydrate) between summer and fall; and the authors suggested that photoperiod may be a potential cue for foraging strategies in ants (Cook et al., 2011).

Some documents were reported that ant food preference did not depend on different seasons. Mashaly et al. (2013) studied the food preference of a Ponerinae ant, *Pachycondyla sennaarensis*, in King Saud University, Kingdom of Saudi Arabia. Due to the fact that the ant completely ignored lipid baits, four baits, which were two protein baits and two carbohydrate baits, were used in this research. The result showed that the ant did not alternate its food preference despite the different seasons.

Eow and Lee (2007) studied the food preference of three *Monomorium* species; *Monomorium pharaonis*, *M. floricola* and *M. destructor* which all of them have been household pests. These three species were fed with various food choices for 24 hours which covered three major nutrients; protein, carbohydrate and lipid. The results showed that *M. floricola* was an oil-loving ant, *M. destructor* was a sugar-loving ant and *M. pharaonis* preferred both protein and oil.

Bait formulations also affect ant food preference. Lee (2008) set sucrose bait base experiment which differed in formulations (gel, liquid, paste and granule) in laboratory with 10 urban pest ant species; *Monomorium pharaonis* (L.), *M. floricola* (Jerdon), *M. destructor* (Jerdon), *M. orientale* Myr, *Tapinoma indicum* (Forel), *T. melanocephalum* (Fabricius), *Anoplolepis gracilipes* (Fr. Smith), *Paratrechina* *longicornis* (Latrielle), *Phidole* sp. and *Solenopsis geminata* (Fabricius). The result showed that all ant species except *Phidole* sp. and *S. geminata* mostly preferred liquid bait, and the researcher described that the indoor nested ant, usually lack of water and moisture, showed preference toward bait with higher moisture content. For *Phidole* sp. and *S. geminata* that not preferred liquid bait resulting from their preference to protein and lipid based diet.

There was a study about the relationship between food preference and an environmental factor. Stein et al. (1990) studied the food preference of red imported fire ant, *Solenopsis invicta*, for a year. This study was carried out in four different habitats; dense forest, lowland pasture, forest with pasture and upland pasture. The baits used in this study were grape agar and tuna fish cat food. The result showed that in all habitat types, red imported fire ant preferred grape agar (carbohydrate source) when the mean soil surface temperatures were low, while the ant preferred tuna fish cat food when the mean soil surface temperatures were high. This can be explained that in the colder temperature, carbohydrates were needed for maintenance. In contrast, in the warmer temperature when the colony growth occurred, proteins were need for the larval development.

2.3.4 Foraging range

Gordon (1995) studied the foraging range of the red harvester ant, *Pogonomyrmex barbatus*. By observing 88 red harvester ant colonies for five years since 1985, so the researcher knew the age of each colony which the red harvester ant colony reached its equilibrium population when age at 5 years old. The results showed the foraging range mostly increased when colony age at about 1-2 years old which this may be the result of colony growth rather than colony size. The researcher still discovered that about the half of foraging range of previous summer was used in the next summer. Because the seeds were not consistent at the same site due to the wind and flooding. Moreover, the surrounding colonies with age specific still influence the foraging range of the studied colonies. The colonies age at 3-4 years old usually conflict with the other colonies because their colonies are larger in size. While the colonies age at 1-2 years old who are smaller in size usually retreat when meet colonies age at 3-4 years old. In contrary, the colonies age at 5 years old or older usually avoid encountering the foreign colonies because colony age at 5 years old have a cost to begin to reproduce sexual forms.

Conspecific competition also influences the ant foraging range. Harrison and Gentry (1981) investigated the foraging range of the Florida harvester ant, *Pogonomyrmex badius* in South Carolina, USA and found that ant colonies were packed together with little overlap. Foraging range also constantly moved toward in the same direction of nest relocation which responded to the movement of proximate colonies. The results suggested that it was not sure if the change of foraging range following nest relocation was a result from other colonies' relocation or seasonal shift. However, the nest relocation would benefit to the colonies because it could reduce the pressure from neighboring colonies and find new food resources.

2.4 Behavioral variation among ant colonies

Ants are well known as social insect. Queen (or queens) and workers live together in the colony which queens rely on workers for food and defense. Colony level is the unit of reproduction and is acted by natural selection pressure. So the differences among colonies could be a crucial path in the evolutionary aspect (Jandt et al., 2014).

There were many studies about the behavioral variation both in intra-colony level and inter-colony level. Not only in the ants but also in the other social animals such as termites, social spiders and especially in bees that showed colonies' variation. The examples of studied behaviors were aggression, boldness, shyness, learning, division of labor, hygienic behavior, foraging, defense, reproduction, cooperation and exploration (Jandt et al., 2014).

Pinter-Wollman (2012) proposed the three hypotheses which shape the colonies differed in personality such as:

- **The average personality of workers**: The colonies vary in the mean worker personality, for example, the aggressive behavior of *Rhytidoponera confuse* was determined by the average aggressive ants, therefore, the more highly aggressive workers, the more overall colony aggression. Due to the fact that the variation in the proportion of older worker was a key role to impose colony aggression because old members in social insect trend to spend more time for guarding and more aggressive than the younger ones (Crosland, 1990).
- **The distribution of worker personalities**: The colonies have differences in distribution of worker personalities. For instance, the first colony consists of few highly active workers and so many workers that are not active and the second colony comprises of all medium level activity of worker. These skews provide a certain advantage to the different situations which lead to variation of behavior. The first colony would benefit when encounter with time-sensitive tasks such as exploit the temporary food source among high competition which need only few active workers. In contrary, the second colony would benefit from the constant work task such as retrieve the food from abundant food source (e.g. seeds) which requires many active workers.
- **The local environment**: Behavioral differences among colonies may vary due to the different local environments or microclimates. For example, the two colonies

which resemble in both average personality and personality distribution may differently exhibit behaviors. If the nest entrance of the first colony is located in more shading area, the worker activity would be lower than another colony which is not always in the shade because the activity correlates with surface temperature (Azcarate et al., 2007).

Later Jandt et al. (2014) illustrated the three mechanisms which cause the variation among colonies.

- **Colony genetics**: The ant queen who mates multiple times or multiple queens in a colony can produce workers with different genetic compositions and lead to differ in behaviors. Although the queen mates only once in its life time (with many males), the genetic variation in workers could arise from the sperm clumping in queen's spermatheca as a consequence of temporal heterogeneity of the workers genetic composition (Wiernasz and Cole, 2010).
- **Colony composition and emergent behavior**: This mentioned mechanism is the combination of the distribution of worker personalities and the distribution of worker personalities as described above by Pinter-Wollman (2012).
- **Colony environment**: As illustrated above, microclimate and microhabitat could influence colony personality. Not only the weather condition (dew point) effects colony behavior but also the terrain such as nest site or nest structure effects the each ant colony behavior. (Pinter-Wollman et al., 2012)

Gordon et al. (2011) studied the variation in foraging activity of harvester ant, *Pogonomyrmex barbatus.* The result showed that each colony differed in baseline rate which patrollers left the colony. Patrollers are the ants which emerge and search the nest mound and foraging trails before other foragers begin to forage in each morning (Gordon, 2002). Moreover, this baseline rate still associated with the colony foraging activity. This can be explained that both patrollers and foragers had the same baseline of activity; therefore, colonies vary in baseline rate of foraging.

Cole et al. (2010) studied the relationship between surface temperature and foraging activity of harvester ant, *Pogonomyrmex occidentalis*. The researchers recorded the temperature at which the ants from each colony started and stopped foraging activity in early summer (June) and late summer (August). There were seasonal differences in foraging patterns and temperature ranges which each colony workers foraged. These associated with workers' physiology and thermal range preference of each colony workers.

Pinter-Wollman et al. (2012) reported that nest site affected personality. The behaviors which require interaction among workers inside the nest were influenced by nest site. The behaviors influencing by nest site in this study were the behavior responding to food bait and alarming. For example, the regulation of foraging activity in *Pogonomyrmex barbatus* depends on the interaction between returning foragers and workers in the nest (Gordon et al., 2008).

2.5 Characteristics of invasive ants

There are three words that are popular used with particular ants so far, tramp ant, invasive ant and pest ant. First, tramp ants mean the ants that have established outside their native ranges with the help of human transportation. Second, invasive ants are the subset of tramp ants which cause ecological, environmental, or economic impacts (Lach et al., 2010; Tsutsui and Suarez, 2003). Finally, pest ants which not all ants are considered as pest at all the time, but they are considered as pest when they cause economic damage to human (Metcalf and Luckmann, 1994).

Many invasive ants share common characteristics which facilitate them to conquer in the new environments. These characteristics are general nesting and diet, polygyny, colony budding and unicoloniality (the consequence is reducing in intraspecific aggression). Although some characteristics mentioned above can be found in non-invasive ants such as general diet and polygyny, here there are some explanations of particular traits in detail (Tsutsui and Suarez, 2003).

- **Polygyny** (multiple queens within a colony) can increase colony growth rate and increase the chance of colony budding.
- **Colony budding** can quickly promote colony fragmentation. For instance, Argentine ants, *Linepithema humile*, usually move their nests to the areas which food resources available or to the environmental sites which are more suitable.
- **Unicoloniality** which the ants reduce the cost of intraspecific aggression and territoriality, ants from different nests (polydomy) can freely move and mix without boundaries. Thus, the ants can directly use the resources to promote colony growth, foraging, resource defense and interspecific competition.

2.5.1 Effect on human

According to the survey conducted during 1983-2001 in Malaysia, it revealed that ants were the third important household pest after mosquitoes and cockroaches, respectively (Lee, 2002). Singapore ants are well known as one of the most common pest ants which usually gnaw holes in fabric, rubber including electric wire insulations which may lead to fire in house and car (Figure 6).The ant also causes in term of property damages and treatments where this ant is abundant (Harris, (n.d.); Smith, 1965). On Tobi Island and Helen Reef Atoll in Palau, Singapore ant causes serious threat to essential infrastructure and causes intensive economic damage in human buildings. The report from Darwin, Australia where Singapore ant causes havoc in the household due to the fact that they bite and present almost everywhere in the house (Wetterer, 2009).

Figure 6 Singapore ants are gnawing electric wire insulation at Biology I Building, Faculty of Science, Chulalongkorn University.

In public health aspect, there were several reports about health threat to human. There was an investigation that found bubonic plague bacteria, *Yersinia pestis*, which cause plague on *T. destructor* feces. There was a report that people were viciously bitten by Singapore ants while sleeping on beds (Smith, 1965). At Loggerhead Key Island in Florida, Singapore ants climbed along the fallen sheet and bitten a new comer scientist while sleeping on a bed until he was unconscious (Wetterer and Espadaler, 2010). Another ant attacking situation was at Cape Verde Island, the residents was bitten by Singapore ants both indoors and outdoors especially the children sleeping at night. Even captive rats still were killed by the ants within 24 hours (Wetterer, 2009).

Although there have not been any reports of the impacts of *T. destructor* on agricultural and horticultural losses, Singapore ants could contaminate in agricultural and horticultural processing plants and, sometimes, cause electrical damage to machinery and processing plants. In glass houses, Singapore ants cause crop contamination and biting gardeners, however, these could be compensated by predation on other invertebrate pests in the glass houses (Harris, (n.d.)).

2.5.2 Effect to ecosystem

There is one concerned case about the invasiveness of *T. destructor* which may threat to the indigenous ecosystem. There was a study about the invasive ants on Baltra Island, Galapagos Archipelago, by collecting ant species from human settlements, airport, garbage dump, dock and less disturbed natural areas. Thirteen ant species were additional found which one of them was *Trichomyrmex destructor*. The ant was found throughout the Baltra Island both natural and inhabited areas though it was first recorded in Galapagos in 1997. However, there have not been any study concerning the impact of Singapore ant to Galapagos (Herrera and Causton, 2010). Notwithstanding, *T. destructor* is accounted as a threat to the native fauna both in Galapagos and Macaronesia (Herrera and Causton, 2010; Wetterer and Espadaler, 2010).

CHAPTER III

Study area

This study was conducted at Faculty of Science, Chulalongkorn University, Bangkok, Thailand during 2014-2015. There were 7 locations in this study (Figure 7). These locations were found by colony survey. The colony survey was conducted by leaving the baits (the mixture of crushed cookie and crushed peanut 1:1 w/w) on plastic pads (8.0 x 8.0 cm) for an hour. Baits were left around locations where ants appeared. After that the plastic pads were picked and put into 70% alcohol, and then the Singapore ants were identified using Bolton, 1994; Bolton, 1995 and Julie and Lee, 2001. Furthermore, the direct observation was also used to find the location of the ant colonies.

Seven Singapore ant colonies, which were different in microhabitats, were found from colony survey (Figure 7). The seven colonies were divided to be used in three different studies:

1. The foraging activity study was conducted at three sites where they were located at (Figure 8):

1.1 SCS: In front of the Faculty of Science sign

SCS colony was located the edge of concrete court in front of the Faculty of Science sign.

1.2 SCL: In front of the Faculty of Science Center for Laboratory Animal Experimentation (SciCLE)

SCL colony was located on the ground between a tree and a concrete court in front of the SciCle building.

1.3 CHL: At one corner of Chakrabongse lawn

CHL colony was located at a crevice of concrete structure at Chakrabongse lawn.

2. The foraging range study was conducted at SCS and SCL sites.

3. Food preference and ant abundance were conducted at 4 sites where they were located at

3.1 TNB: Tab Nilaniti Building

TNB colony was located at the crevice of Tab Nilaniti Building's corner. This colony was shaded throughout the day.

3.2 ENG: The corner which is opposite to Faculty of Engineering

ENG colony was located at the holes of a withered tree trunk which is opposite to Faculty of Engineering.

3.3 CHB: In front of Chakrabongse Building

CHB colony was located at the edge of concrete footpath which was adjacent to a concrete road in front of Chakrabongse Building.

3.4 CML: Yard in front of Chemistry II Building

CML colony was located on the ground which was adjacent to tree's buttresses.

Figure 7 The seven study sites at Faculty of Science, Chulalongkorn University (modified from Google map). SCS) Faculty of Science sign, SCL) SciCLE Building, CHL) Chakrabongse lawn, TNB) Tab Nilaniti Building, ENG) The corner opposite to Faculty of Engineering, CHB) In front of Chakrabongse Building and CML) Yard in front of Chemistry II Building

Figure 8 The study sites. SCS) Faculty of Science sign, SCL) SciCLE Building, CHL) Chakrabongse lawn, TNB) Tab Nilaniti Building, ENG) The corner opposite to Faculty of Engineering, CHB) Chakrabongse Building and CML) Yard in front of Chemistry II Building.

CHAPTER IV

Foraging activity and foraging range

4.1 Introduction

Trichomyrmex destructor or Singapore ant is well known as one of the common pests in the urban environment. It causes nuisances and damages especially to household and some serious health aspects (Harris, (n.d.); Smith, 1965; Wetterer, 2009; Wetterer and Espadaler, 2010). Singapore ant posts a polygynous character (many queens in a colony), so this ant species can quickly increase its population in a short period of time (Debout et al., 2007).

The study of foraging activity of particular pest ants would provide us the information to conduct the better and more accurate monitoring program. However, only few studies of foraging activity and foraging range of *T. destructor* have been conducted especially under the field condition and in full-year scale. The first purpose of the study is to examine whether Singapore ants show different foraging activities in the urban environment according to the seasonal differences in both the presence and absence of bait conditions. The second aim is to investigate the foraging range of Singapore ant all year round if there are any differences due to the different seasons.

4.2 Preliminary study (Bait selection)

A preliminary study was carried out to find the most preferred food type by using various types of bait based on nutrition used as a bait to study foraging activity and to find the ant colony locations for the study of foraging activity, foraging range and ant abundance.

Three ant colonies were once used in this preliminary study which consisted of colonies namely CHL (Chakrabongse lawn), SCS (Faculty of Science sign) and SCL (Faculty of Science Center for Laboratory Animal Experimentation). The preliminary study was conducted by using 5 bait types which were crushed cookie, honey, strawberry jam, crushed peanut and canned tuna in salt water (Figure 9). Each bait type was placed separately in a plastic petri dishes and left for half an hour. Then, each plastic petri dish was kept in a plastic zip-lock bag and frozen before counting the number of ants. Before performing statistical analysis, the data were checked for normality and homogeneity of variance. The result showed that our data violated parametric criteria, so Kruskal-Wallis test was used to find the significant difference of ant number for all bait types, and then Man–Whitney U test was performed to find out the differences of ant number of each bait pair.

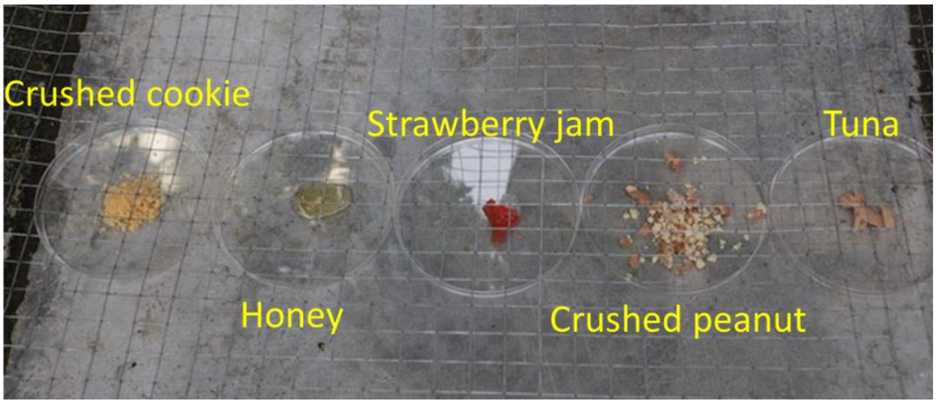

Figure 9 The five bait types that were used as baits in a preliminary study.

The mean (± SE) number of ant of each bait is shown in Table 1. The result showed that there was a significant difference among the bait types (Kruskal-Wallis: X^2 = 9.631, df = 4, *p-value* = 0.047), and the result from Man-Whitney U test showed that honey and jam were less attractive than other kind of baits (Table 2). Thus, crushed cookie, crushed peanut and canned tuna were the most preference food. However, for the ease to formulate and preserve the bait, only crushed cookie and crushed peanut were selected to use as the bait for colony survey and for foraging activity study.

Table 1 The means ± SE of ant number/trap from a plastic petri dish of each bait (crushed peanut, crushed cookie, honey, jam and tuna)

Bait	Mean ± SE (individual/trap)
Crushed peanut	149 ± 74.57^{ab}
Crushed cookie	204 ± 53.89^{a}
Honey	17.33 ± 6.64^{b}
Jam	6 ± 6.00^{b}
Tuna	78.33 ± 60.05^{ab}

Note: Different letters designate statistical significances of mean numbers.

Table 2 The significant differences of the ant numbers caught by baiting in each paired bait type (Man-Whitney U test, $p < 0.05$)

Paired baits	*P-value*
Peanut-Cookie	0.564
Peanut-Honey	0.189
Peanut-Jam	0.091
Peanut-Tuna	0.384
Cookie-Honey	0.020**
Cookie-Jam	0.018**
Cookie-Tuna	0.081
Honey-Jam	0.439
Honey-Tuna	0.559
Jam-Tuna	0.074

** Significant difference at $p < 0.05$

4.3 Materials and Methods

4.3.1 Daily foraging activity

During 12 months (December 2014 – November 2015), three ant colonies were studied consisting of colonies named SCS, SCL and CHL. In foraging activity study, there were two treatments which were the regular ant activity (without bait) and the ant activity with bait (with bait) responding to the excess stimulant (bait: the mixture of crushed cookie and crushed peanut 1:1 w/w). The activity was recorded as the number of returning ants (the ants that traveled back to the nest) walking across an imaginary line in 1 minute (returning ant number/minute). This imaginary line was the line drawn out of a pencil tip (Figure 10). Only the returning ants were counted because they showed the rate of outgoing ant and food availability (Gordon et al., 2013). Each treatment was conducted two replicates/month and the data of each replicate were recorded every two hours for 24 hours. In addition, the count was also conducted every half an hour from late morning to midday (around 10 am to 12 pm) and from late afternoon to early evening (around 3.30 pm to 6 pm) when it was the peak of ant activity. The counts were conducted around each nest entrance (approximately 60 cm from nest entrance) of the three ant colonies by using a mechanical counter. A mechanical counter was used for counting the low abundance that could be counted with the naked eyes. For the high ant abundance, an iPhone 6 mobile phone was used to record the video of the returning ants for 1 minute. Then the returning ants (in VDO file) were counted in a computer with reduced play speed.

Figure 10 The imaginary line drawn out of a pencil tip.

In ant activity with bait treatment, the bait was placed on the one edge of a box-shaped plastic station with removable lid laying paralleled to the ant trail (Figure 11). The size of plastic station base is 15 cm in length and 15 cm in width (15 cm x 15 cm). The two sides (wall of the box) are 15 cm in width and 8 cm in height (15 cm x 8 cm). The other two sides are 15 cm in width and 4 cm in height (15 cm x 4 cm) which located on the center of the side. Each station was located approximately 60 centimeters from the nest entrance.

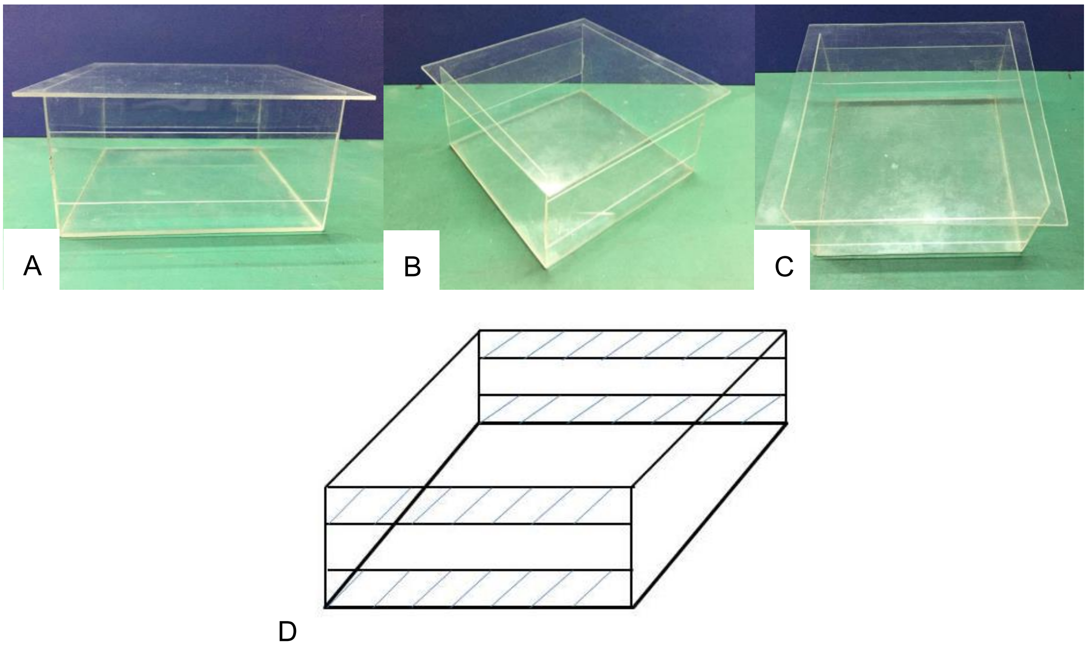

Figure 11 The plastic station with a removable lid. A) Front view B) Oblique view C) Top view and D) The drawn station with the open areas (shaded areas)

During the ant counting in both treatments (regular ant activity and ant activity with bait), the ambient temperature and relative humidity were measured by hygrometer (Mason's hygrometer, Brannan), and surface temperature was measured by infrared thermometer (Raytek MiniTemp MT2) (Figure 12).

Figure 12 Mason's hygrometer (left) and infrared thermometer (right)

The ant behaviors such as foraging behavior and interspecific interaction were also observed and recorded both in images and VDOs media.

4.3.2 Foraging range

The two ant colonies, SCS (Faculty of Science sign) and SCL (Faculty of Science Center for Laboratory Animal Experimentation) were used in this study from December 2014 to November 2015. Each month, the foraging ranges of ten ants of each colony were measured by measuring tape. In this study, the foraging range was the distance between nest entrance and the farthest point which ants had reached before the ant returned to the nest entrance. Normally, Singapore ants have formed the trail out of its nest entrance, but in this study, the individual ant which did not follow the existent trail was used as a model to measure foraging range.

4.4 Data analysis

4.4.1 Determination of season

To discriminate the different seasons (wet and dry seasons), the total rainfall (mm) and the mean temperature (°C) in each month were used to generate the bar chart using the scale of 10°C corresponding to 20 mm of precipitation. The months with the bar of precipitation above the line of mean temperature were accounted to be in wet season and the months with the bar of precipitation below the line of mean temperature were accounted to be in dry season (Walter et al., 1975). The climatological data were obtained from the Thai Meteorological Department.

4.4.2 Statistical analysis

Data were checked for normality and equal variances before conducting further analyses. If the data did not meet the assumptions for parametric analyses, the non-parametric analyses were then applied.

4.2.2.1 Daily foraging activity

The foraging activity data of the three ant colonies were pooled together before conducting statistical analysis. Ant activity data at daytime and nighttime year-round were compared in both regular ant activity and ant activity with bait. Only the daytime activity data were used to compare between the regular ant activity and ant activity with bait. To determine the effect of different seasons on ant activity, daytime data were compared between wet and dry seasons, and between regular ant activity and ant activity with bait. Statistical tests in this part were conducted using the SPSS software (version 17.0).

4.2.2.2 Correlation and relationship between foraging activity and environmental factors

To see the overall trends of each physical factor on ant activity and to analyze the correlation between ant activity and physical factors, the ant activity data from the three ant colonies (SCS, SCL and CHL) during daytime and nighttime were merged together. Then, the returning numbers of ants/minute (zero excluded) were plotted against each physical factor (surface temperature, relative humidity and air temperature) both in wet and dry seasons. The differences of physical factors between wet and dry seasons were compared using the SPSS software (version 17.0).

Since the response variable (ant number/minute) were discrete data, Poisson regression was suitable to analyze the relationships between the returning ant numbers (pooled data from regular ant activity and ant activity with bait) and the three physical factors. However, the data encountered with over-dispersion (variance larger than the mean: ϕ (dispersion parameter) > 1) (Rodr´ıguez, 2013), other methods dealing with over-dispersion were used including quasi-Poisson model, negative binomial model, hurdle model and zero-inflated model (Zeileis et al., 2008) which hurdle model was capable to deal with only sampling zeros while zero-inflated model could deal with structural and sampling zeros (Hu et al., 2011).

In addition, species response curves (regular ant activity and ant activity with bait) were generated to find the optimum points and environmental tolerance values (Holland, 2014) based on the most suitable model. There are three parameters in species response curve: environmental tolerance (ET), preferred environment (PE) or optimum point and peak abundance (PA) as shown in Figure 13.

Environmental tolerance (ET) is a range which organism is found along the environmental gradient. Preferred environment (PE) is the position which organism is most likely to be found in term of the highest abundance. Peak abundance (PA) is the maximum number of organism which can be found at preferred environment (Holland and Zaffos, 2011). The statistical tests in this part were conducted using the R software (version 3.3.0).

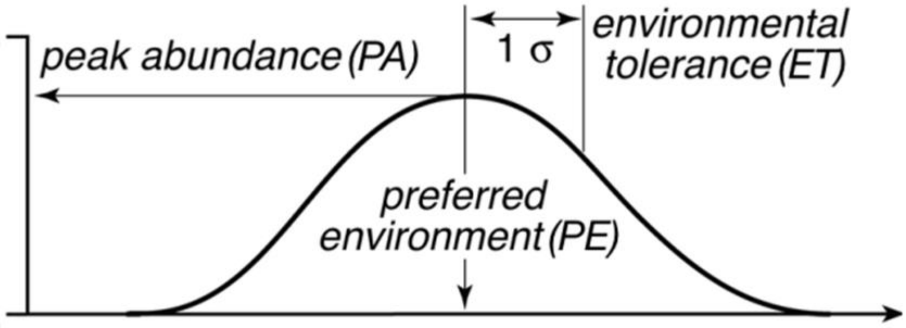

Figure 13 The graph represents species response curve and three parameters, environmental tolerance (ET), preferred environment (PE) and peak abundance (PA) (Holland, 2014).

4.2.2.3 Foraging range

The range data of two colonies were combined and then calculated for the difference of foraging ranges between dry and wet seasons. The mean values and standard error of means of the two seasons were also reported. Statistical tests in this part were conducted using the SPSS software (version 17.0).

4.3 Results

4.3.1 Daily foraging activity

The average foraging activity of the three Singapore ant colonies conducted for 12 consecutive months revealed that the peak activity of *T. destructor* occurred from 4 pm to 6 pm, and the average ant number (mean ± SE) in foraging activity during daytime (31.44 ± 1.53 ants/minute) was greater than during nighttime (10.77 ± 0.95 ants/minute) (Mann-Whitney U test: U = 37836, $n_1 = 422$, $n_2 = 404$, $p\text{-}value < 0.001$) (Figure 14). Moreover, the initiation and cessation of foraging activity of Singapore ant was not affected by the sunrise and sunset times (Figure 15). Singapore ant tended to forage throughout the day except some periods when there was no worker foraging at all (Figure 15, D and K).

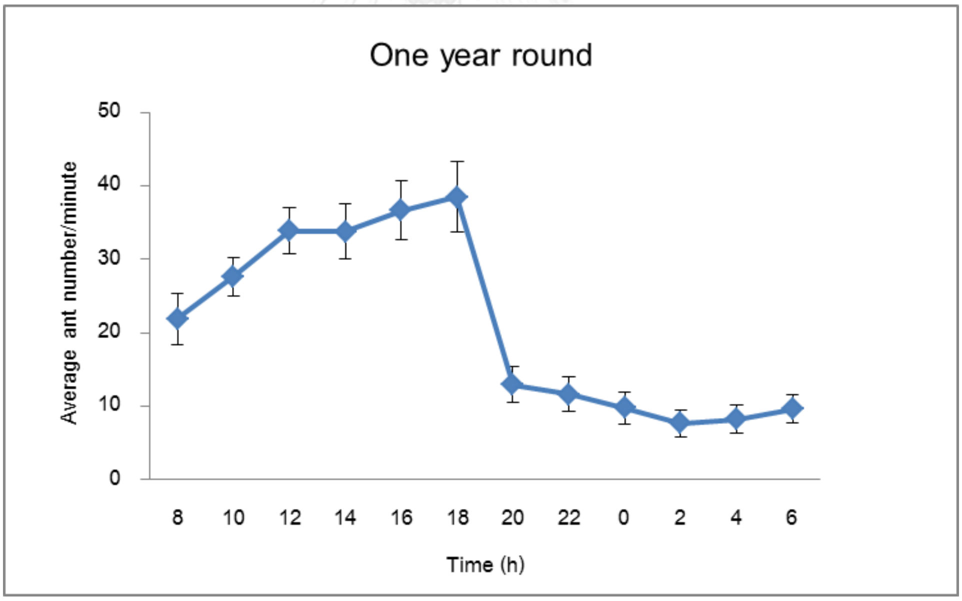

Figure 14 The average daily foraging activities (means ± SE) of returning ant number/ minute of the three Singapore ant colonies (SCS, SCL and CHL) throughout one year during December 2014 – November 2015.

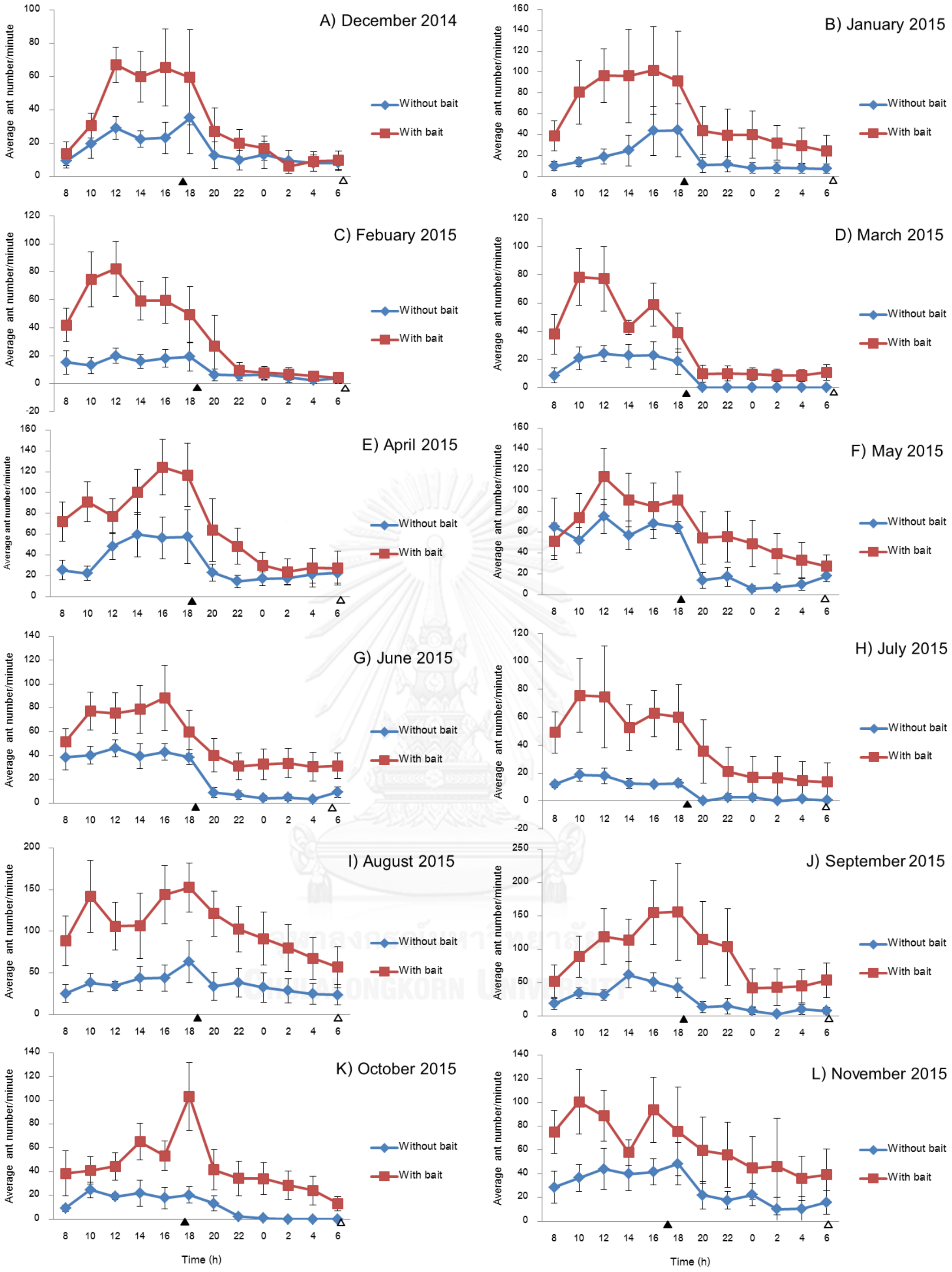

Figure 15 The average daily foraging activities (means ± SE) of returning ant number/minute of the three Singapore ant colonies (SCS, SCL and CHL) in one year from December 2014 to November 2015. The solid triangles indicate sunset time whereas the open triangles indicate sunrise time.

Since the regular foraging activity and foraging activity with bait data did not meet parametric criteria, then Mann-Whitney U test were applied. The average ant number (mean ± SE) in foraging activity with bait (75.41 ± 3.18 ants/minute) was significantly higher than the regular foraging activity (31.44 ± 1.53 ants/minute) (Mann-Whitney U test: U = 49656, n_1 = 422, n_2 = 429, *p-value* < 0.001). The ant foraging activity with bait rose in the morning, dropped down in the afternoon and then increased again in the late afternoon (Figure 16).

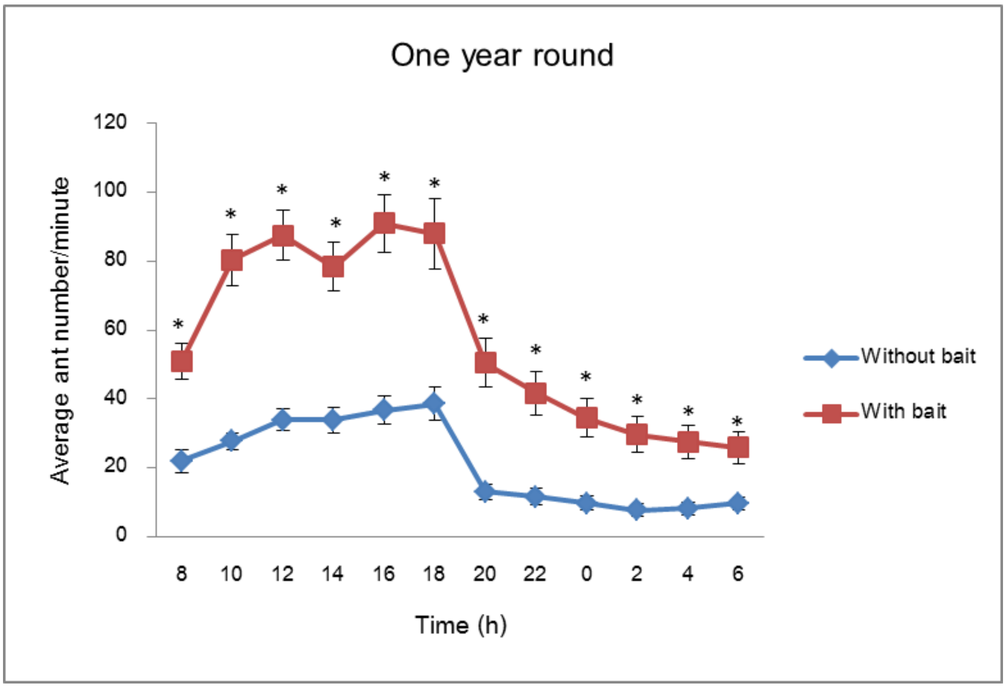

Figure 16 The average daily foraging activities (means ± SE) of returning ant number/minute of the three Singapore ant colonies (SCS, SCL and CHL) throughout one year during December 2014 – November 2015

Note: Asterisks indicate significant difference between paired hours (without bait and with bait) (Mann-Whitney U test: $p < 0.05$).

During the one year period of study, the two seasons were discriminated, dry season (December 2014 – February 2015 and November 2015) and wet season (March 2015 – October 2015) according to the determination of season mentioned in data analysis (Figure 17).

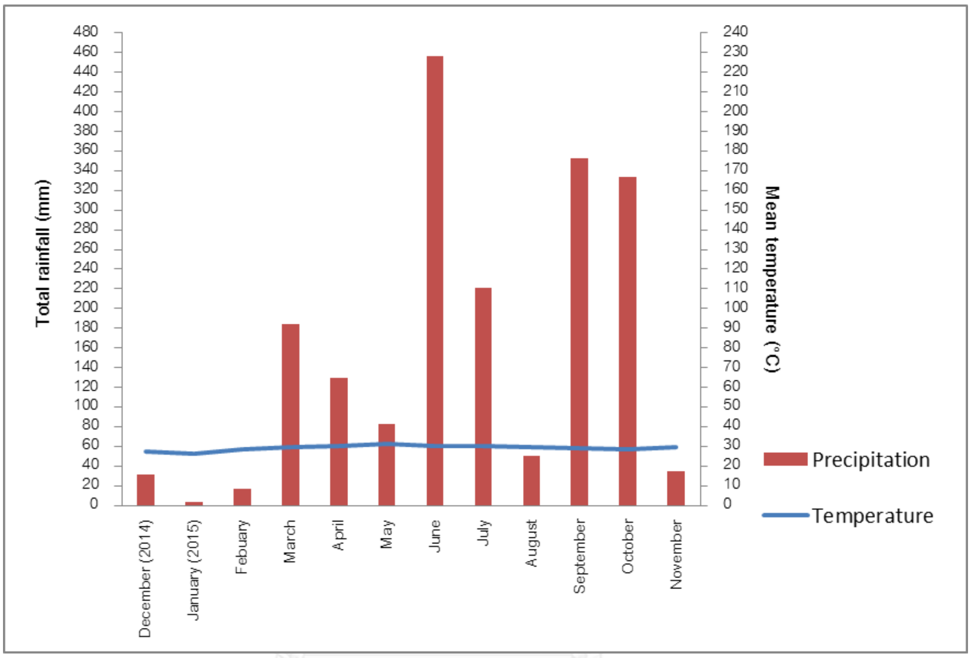

Figure 17 The plotted bar chart and line graph between the total rainfall and the mean temperature of each month for 12 months

The ant foraging activity data were divided into two seasons, dry and wet seasons. The data of ant activities in dry and wet seasons violated parametric criteria, so Mann-Whitney U test was used. The overall results showed the average ant number (mean ± SE) in foraging activity (both regular activity and activity with bait) in wet season (56.38 ± 2.37 ants/minute) was significantly higher than dry season (47.18 ± 3.22 ants/minute) (Mann-Whitney U test: U = 68390.5, n_1 = 256, n_2 = 595, *p-value* = 0.018).

Likewise, the average ant number (mean ± SE) in foraging activity with bait in dry season (68.50 ± 5.20 ants/minute) was significantly greater than the regular foraging activity in dry season (24.84 ± 2.49 ants/minute) (Mann-Whitney U test: $U = 3715$, $n_1 = 125$, $n_2 = 131$, *p-value* < 0.001). The average ant number (mean ± SE) in foraging activity with bait in wet season (78.46 ± 4.00 ants/minute) was also significantly greater than the regular foraging activity in wet season (34.22 ± 1.88 ants/minute) (Mann-Whitney U test: $U = 25680$, $n_1 = 297$, $n_2 = 298$, *p-value* < 0.001) (Figure18). Paired hours both regular activity without bait and activity with bait in dry season were compared and the results showed that only daytime activity with bait (8:00 a.m. to 16.00 p.m. for dry season) was significant greater than daytime activity without bait (Mann-Whitney U test: $p < 0.05$). While the results of paired hours both activity without bait and activity with bait in wet season showed that activity with bait was significant greater than activity without bait (Mann-Whitney U test: $p < 0.05$) (Figure 18).

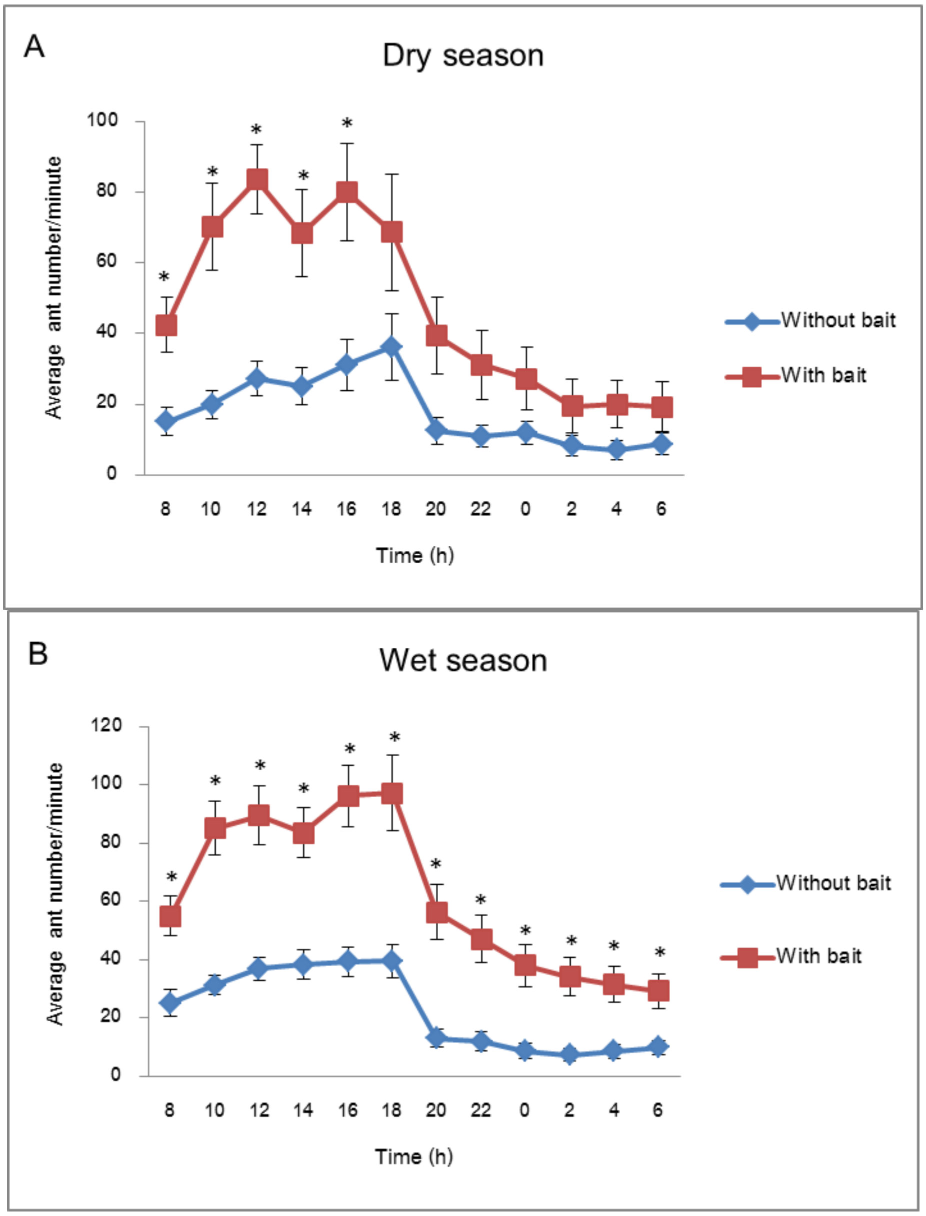

Figure 18 The average daily foraging activities (mean ± SE) of returning ant number/minute of the three Singapore ant colonies (SCS, SCL and CHL) in A) dry season (December 2014, January 2015 to February 2015 and November 2015) and in B) wet season (March 2015 to October 2015).

Note: Asterisks indicate significant difference between paired hours (without bait and with bait) (Mann-Whitney U test: $p < 0.05$).

Because surface temperature, relative humidity and air temperature data did not meet parametric criteria, Mann-Whitney U test was applied. In detail, there were some differences of ant activities between wet and dry seasons, and there were significant differences of each physical factor between wet and dry seasons (Table 3) (Surface temperature; U = 332078.5, n_1 = 694, n_2 = 1489, *p-value* < 0.001; Relative humidity; U = 339847, n_1 = 694, n_2 = 1489, *p-value* < 0.001; Air temperature; U = 246034, n_1 = 694, n_2 = 1489, *p-value* < 0.001). The ants tended to forage under higher surface temperature, air temperature and relative humidity in wet season (Figure 19). This result was consistent with the result from Figure 17 that there were more foraging ants in wet season.

Table 3 The mean (± SD) of surface temperature, relative humidity and air temperature in wet and dry seasons. (Mann-Whitney U test, $p < 0.05$)

Physical factors	Mean ± SD	
	Wet	Dry
Surface temperature	31.77±0.08°C^a	29.79±0.17°C^b
Relative humidity	68.54±0.28%^{a}	61.48±0.47%^{b}
Air temperature	31.50±0.06°C^a	28.77±0.11°C^b

Note: Different letters in the same row designate significant difference at $p < 0.05$.

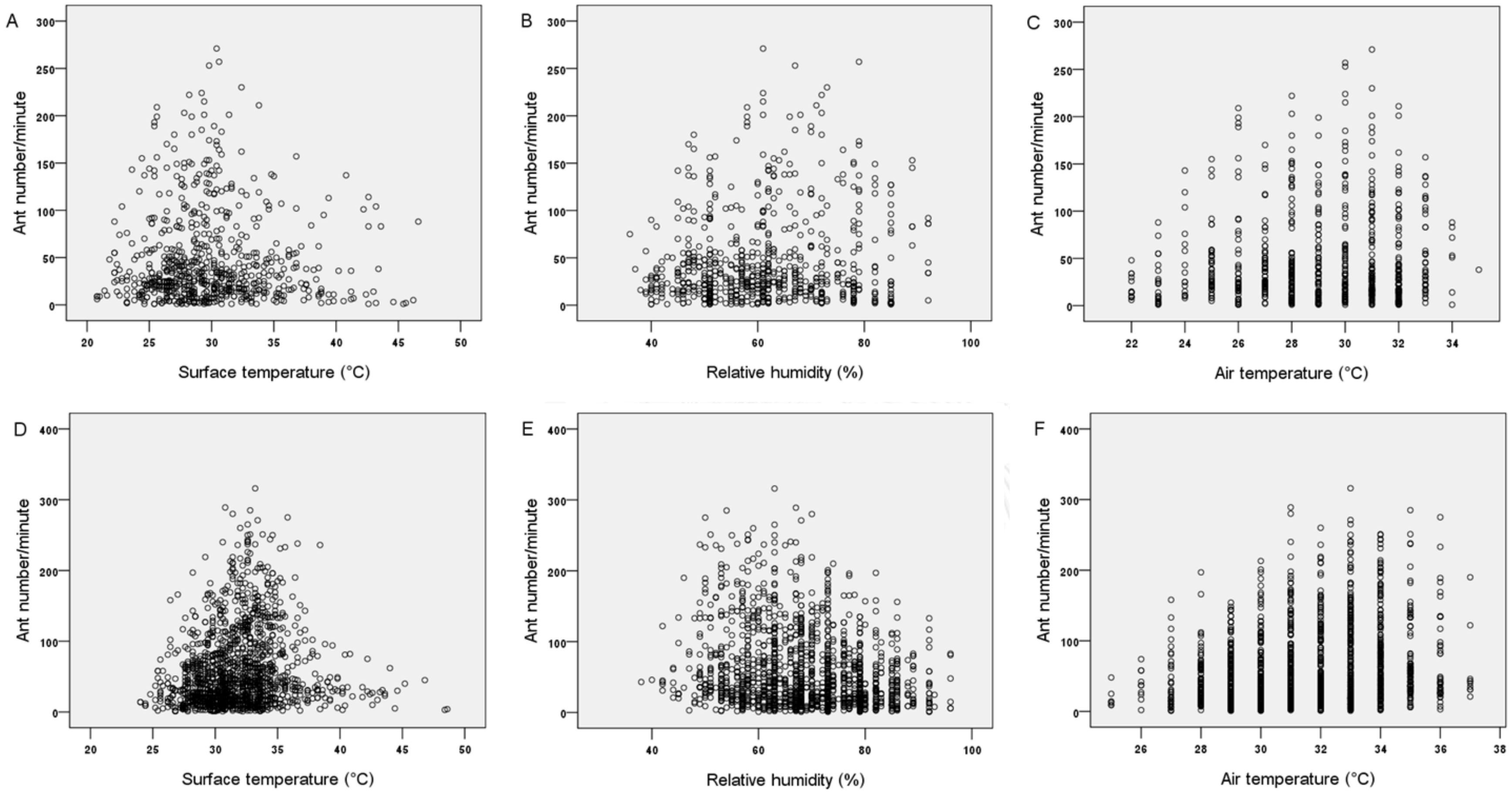

Figure 19 The scatter plots of raw data of the returning ant numbers/minute during December 2014– November 2015 against surface temperature, relative humidity and air temperature in dry season (A,B and C) and wet season (D,E and F).

Actually, in this study, the three different colonies exhibited various foraging behavior patterns. The Singapore ant showed the variation in foraging patterns in all year round, wet season and dry season (Figure 20–22).

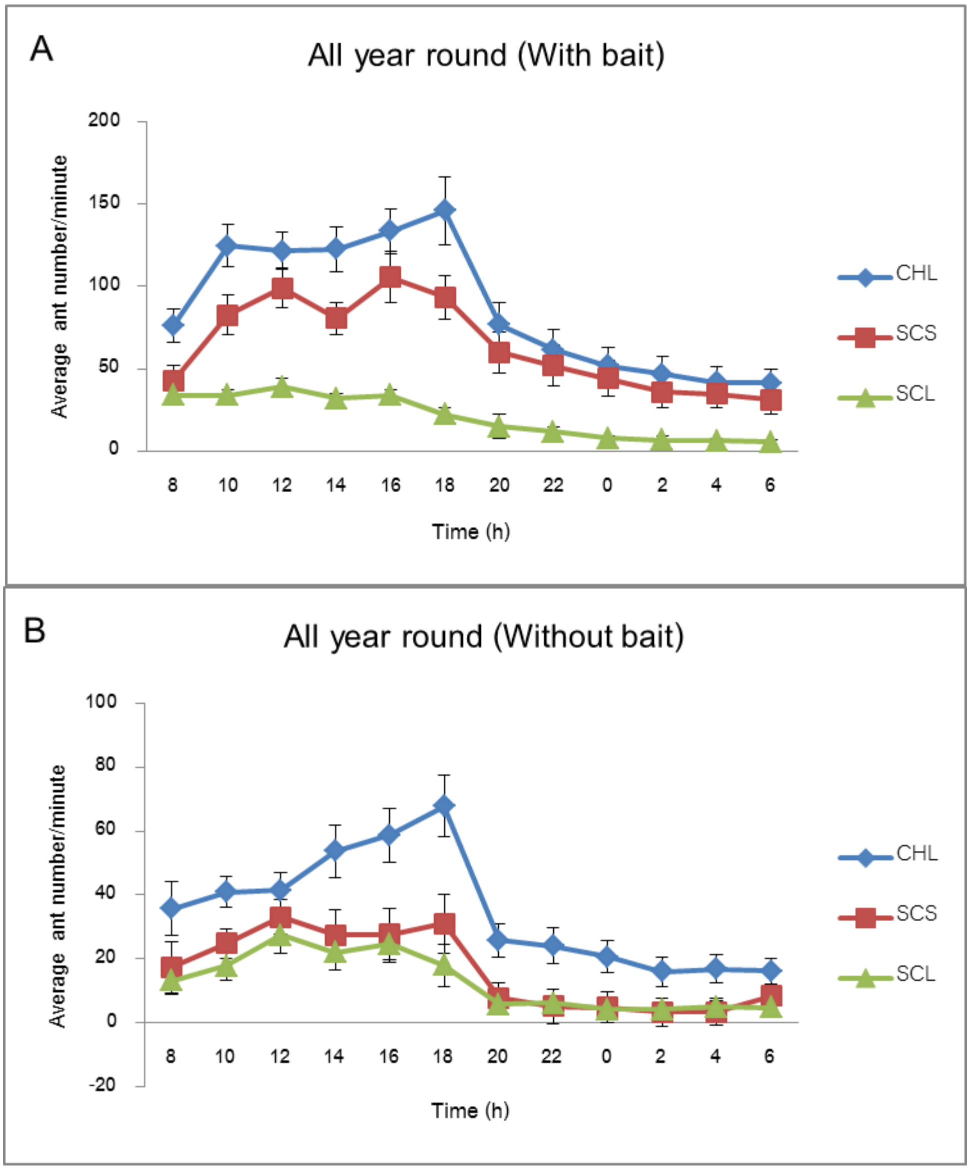

Figure 20 The variation of foraging patterns of three Singapore ant colonies named SCS, SCL and CHL from December 2014 to November 2015. A) All year round (With bait) and B) All year round (Without bait)

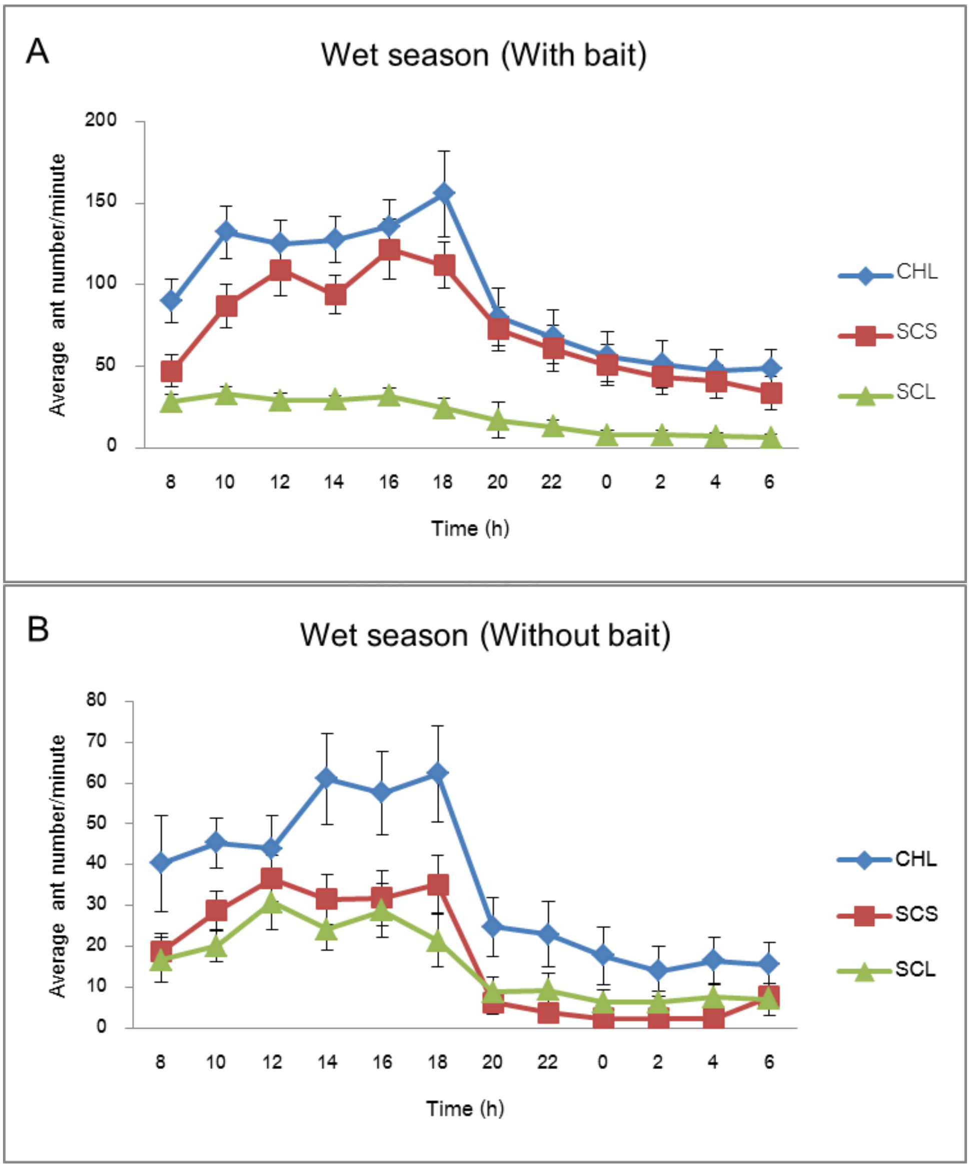

Figure 21 The variation of foraging patterns of three Singapore ant colonies named SCS, SCL and CHL from March 2015 to October 2015. A) Wet season (With bait) and B) Wet season (Without bait)

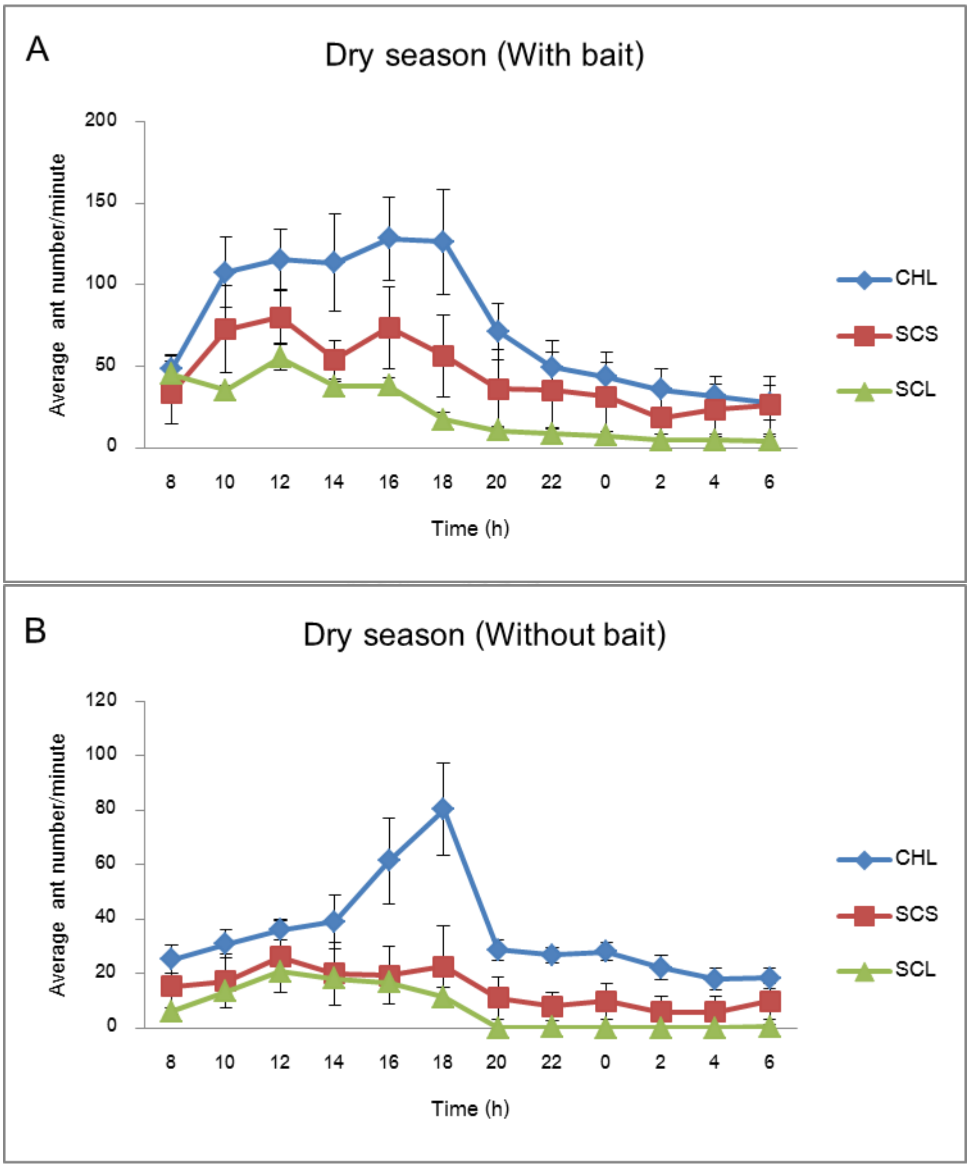

Figure 22 The variation of foraging patterns of three Singapore ant colonies named SCS, SCL and CHL from December 2014, January 2015 to February 2015 and November 2015. A) Dry season (With bait) and B) dry season (Without bait)

4.3.2 Correlation and relationship between foraging activity and environmental factors

The collected environmental factors in this study were surface temperature, ambient temperature and relative humidity. To find the correlation between these physical factors and the numbers of returning ant, the scatter plots were plotted between each factor and the numbers of returning ants in 12 consecutive months from December 2014 to November 2015 (Figure 23). From the scatter plots, the data obviously exhibited the curvy line, so it is not legitimate to proceed neither Pearson correlation nor Spearman correlation (Hawkins, 2005).

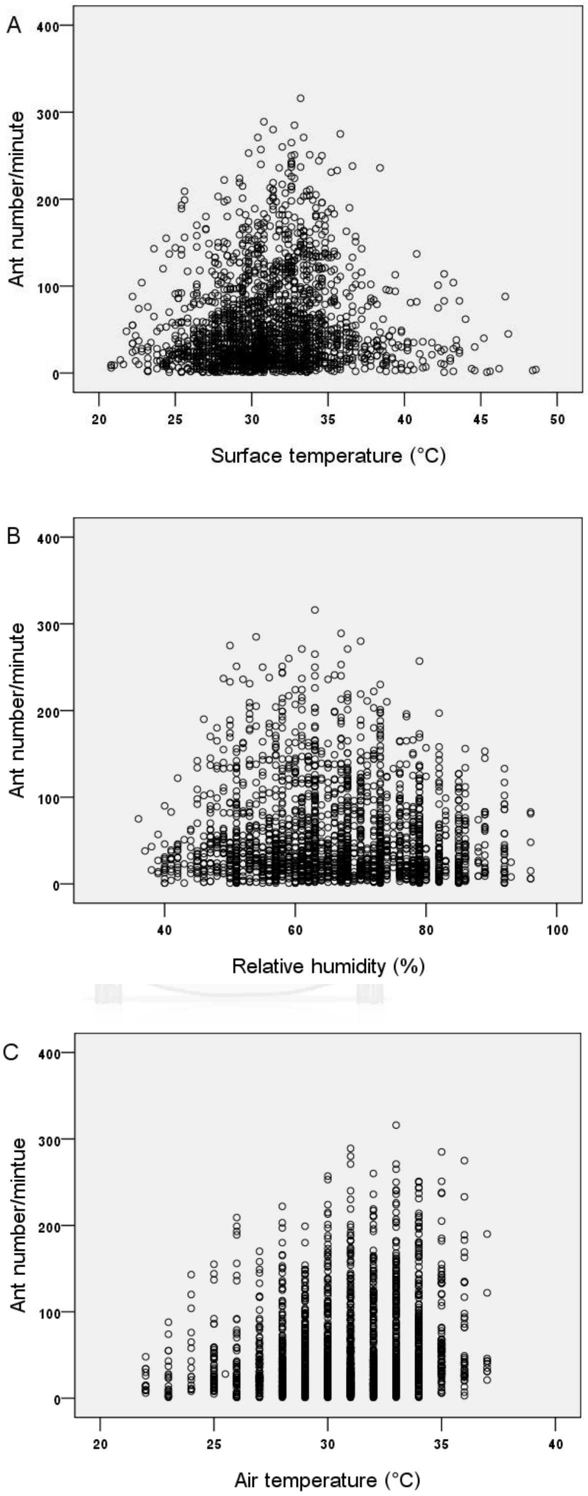

Figure 23 The returning ant number/minute in 12 months (December 2014-November 2015) was plotted against A) surface temperature, B) relative humidity and C) air temperature.

The result from Poisson regression showed that ant activity data encountered over-dispersion (dispersion parameter (ϕ) = 58.07, *p-value* < 0.001) which violated the equidispersion (variance and mean are equal) assumption of Poisson regression. Then, quasi-Poisson, negative binomial model, hurdle model and zero-inflated model were applied. The results of these four models were reported in Table 4.

Table 4 The coefficient estimates from quasi-Poisson, negative binomial model, hurdle model and zero-inflated model (with standard errors in parentheses), maximized log-likelihood and AIC for regular ant activity.

Distribution Method Object	Quasi-Poisson	Negative binomial model	Hurdle model	Zero-inflated model
Intercept	0.1136 (0.397)	0.3536 (0.420)	-4.9775 (0.717)	-4.9775 (0.717)
Surface temperature	-0.0608*** (0.011)	-0.0708*** (0.012)	-0.0816** (0.027)	-0.0816** (0.027)
Relative humidity	-0.0043 (0.002)	-0.0074** (0.003)	-0.0418*** (0.005)	-0.0418*** (0.005)
Air temperature	0.1905*** (0.014)	0.1996*** (0.016)	0.3988*** (0.036)	0.3988*** (0.036)
log L	–	-12911.79 (df=5)	-56128.48 (df=8)	-56128.48 (df=8)
AIC	–	25833.58	112273	112273

*** Variable is significant at the level of *p-value* < 0.001.

** Variable is significant at the level of *p-value* < 0.05.

The results from Table 3 revealed that negative binomial model was the most appropriate model in terms of Log likelihood and AIC (Akaike information criterion) due to the maximum value of Log likelihood and the minimum value of AIC. However, quasi-Poisson could not produce both Log likelihood and AIC. Therefore, mean-variance relationship graph (Figure 24) was generated to elucidate which model between quasi-Poisson and negative binomial models could better explain variance among the increasing means.

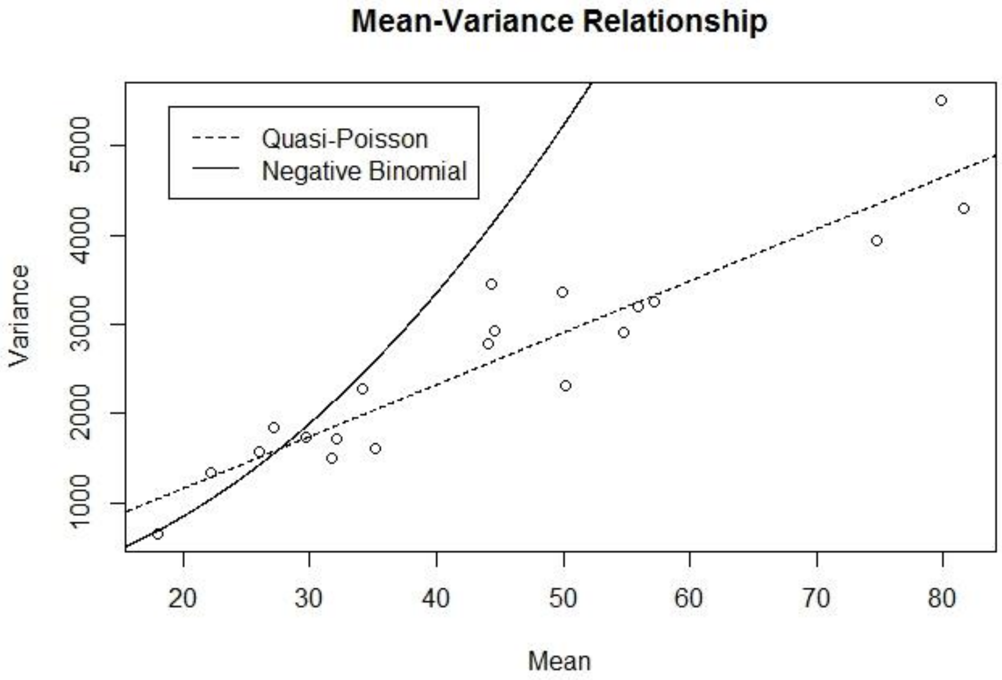

Figure 24 The mean-variance relationship. Solid line indicates negative binomial model while dot line indicates quasi-Poisson model.

From Figure 23, it was obvious that quasi-Poisson was better than negative binomial model to explain the variance. This was because quasi-Poisson variance was a linear function of the mean while negative binomial variance was a quadratic function of the mean. Therefore, the relationship between the number of returning ants and physical factors would be interpreted based on quasi-Poisson.

From the quasi-Poisson model, the number of returning ants was significantly influenced by both surface temperature and air temperature (*p-value* < 0.001), while humidity was not significant (*p-value* = 0.0536).

For species response curves, surface temperature and relative humidity in both regular ant activity and ant activity with bait were generated based on quasi-Poisson (Figure 25-26), but air temperature could not be generated by species response curve because the narrow range of X axis (air temperature) could not reflex the whole ant response according to the change of air temperature. Thus, the response curves of air temperature were likely to direct variation to the increasing air temperature which was the misleading interpretation (Figure 27). Three parameters (including 95% confident interval); environmental tolerance (ET), preferred environment (PE) and peak abundance (PA) were reported in Table 5.

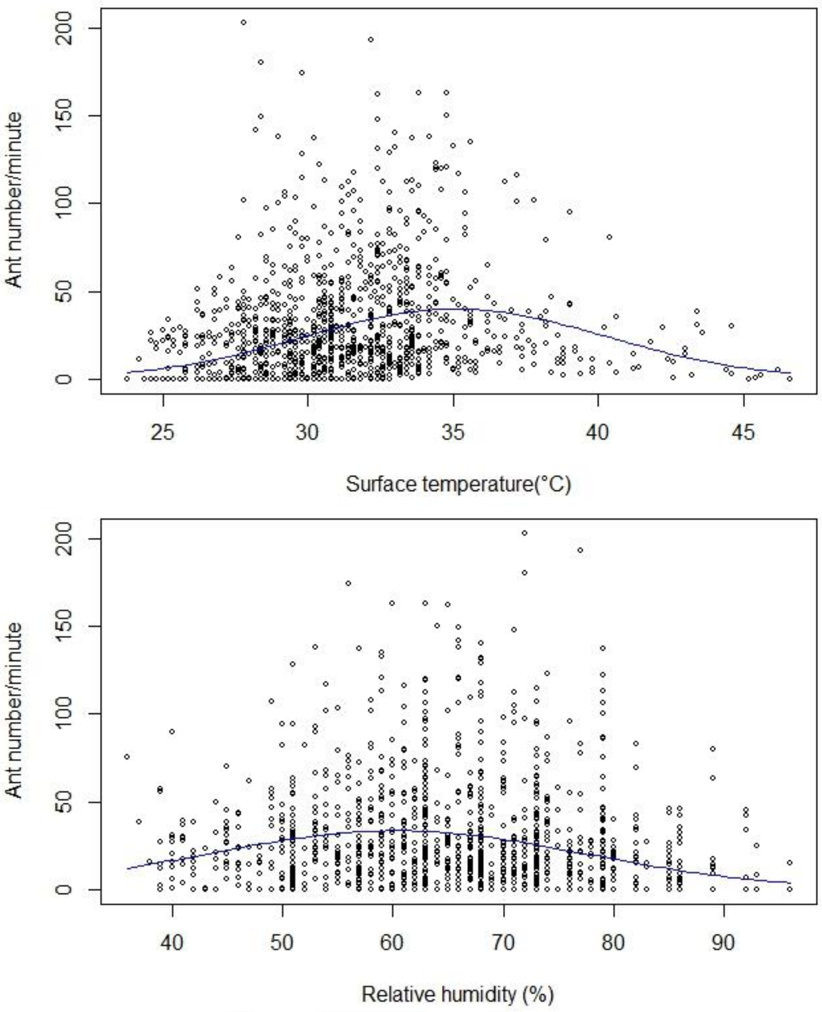

Figure 25 The scatter plots of raw data of returning ant number/minute and foraging activity response curves of Singapore ant (without bait) during December 2014 – November 2015, plotted against surface temperature and relative humidity.

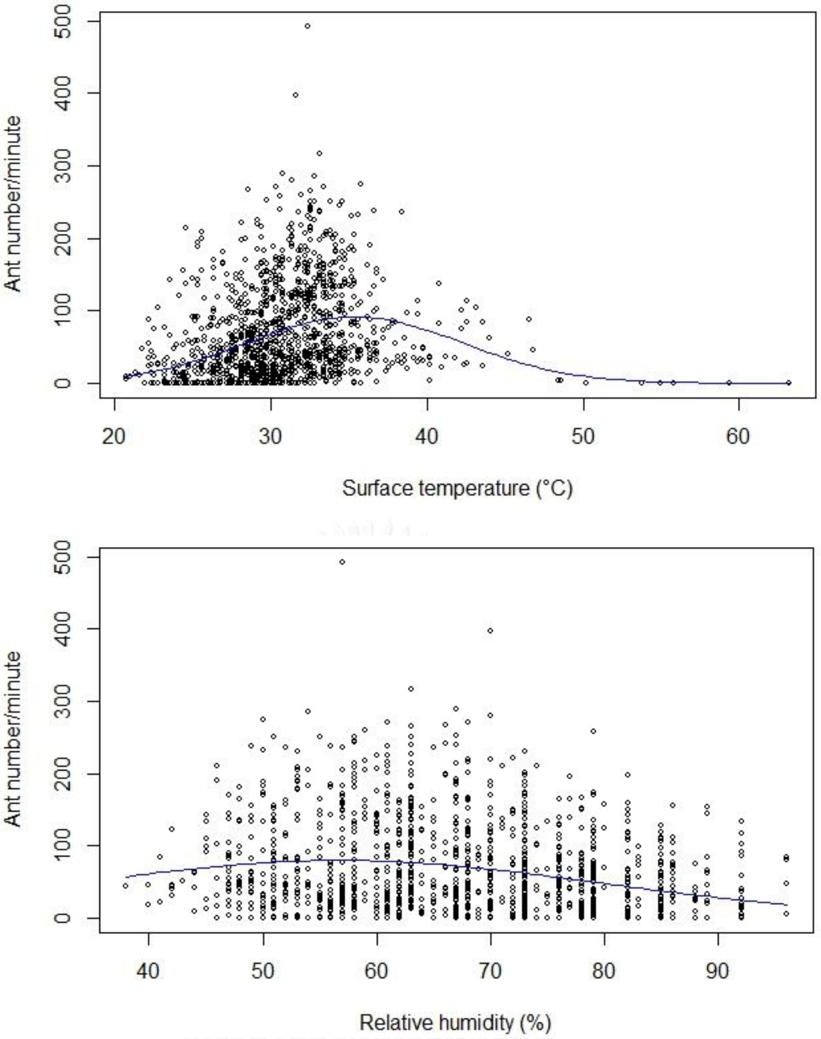

Figure 26 The scatter plots of raw data of returning ant number/minute and foraging activity response curves of Singapore ant (with bait) during December 2014 –November 2015, plotted against surface temperature and relative humidity.

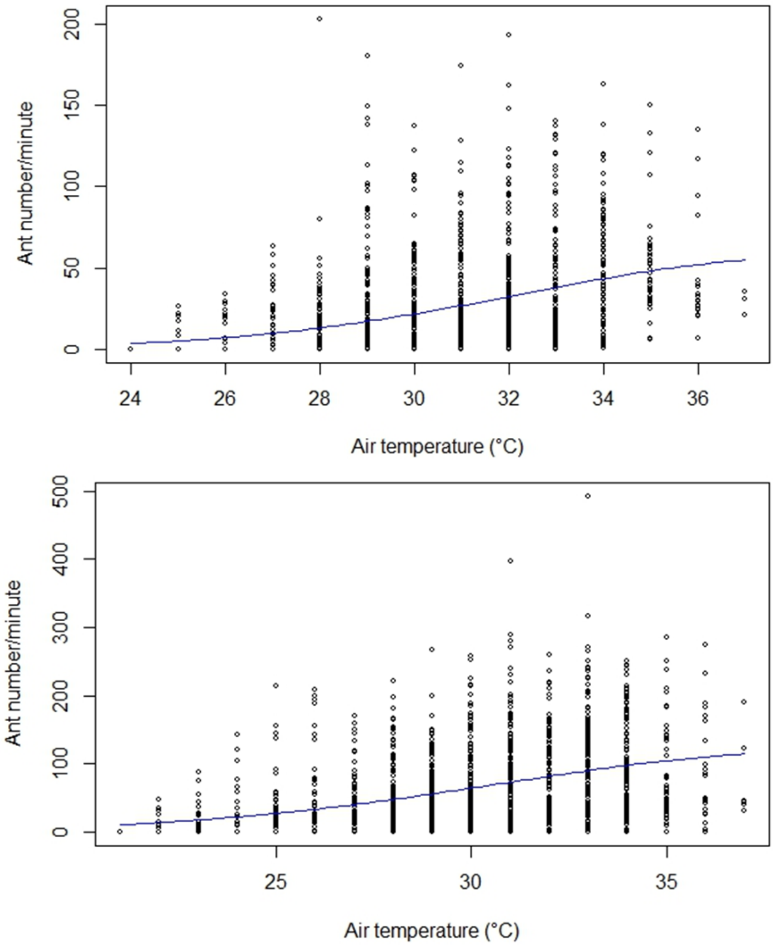

Figure 27 The scatter plots of raw data of returning ant number/minute and foraging activity response curves of Singapore ant (upper: without bait, lower: with bait) during December 2014 – November 2015, plotted against air temperature.

Table 5 Summary of the effects of three parameters, environmental tolerance (ET), preferred environment (PE) and peak abundance (PA) according to surface temperature and relative humidity both in regular ant activity and ant activity with bait

	Physical factors	Environmental tolerance (ET)	Preferred environment (PE)	Peak abundance (PA) (individuals)
Without bait	Surface temperature	29.89–40.25°C	35.07°C	40.12
	Relative humidity	43.26–77.38%	60.32%	33.56
With bait	Surface temperature	28.47–42.31°C	35.39°C	91.32
	Relative humidity	33.78–79.74%	56.76%	79.77

From Table 5, the result showed that environmental tolerance intervals (surface temperature and air temperature) of Singapore ant activity with bait were wider than activity without bait. Singapore ant activity with bait was slightly more tolerant to the increased surface temperature and the lower relative humidity.

In addition, when the numbers of ants were plotted against physical factor data in different bait conditions (regular ant activity and ant activity with bait), the results were consistent with the results from species response curves that the workers were likely to forage in the wider range of physical factors when the bait was appeared (Figure 28). It seems that the ants were likely to forage further from their favored physical range when the food source emerged. For example, the ants in regular ant activity never foraged when the surface temperature closed to 20°C or when the ambient temperature dropped below 25°C. Conversely, some workers in ant activity with bait still foraged when surface temperature closed to 50°C. However, the relative humidity seemed to have no effects on the wider foraging range in ant activity with bait.

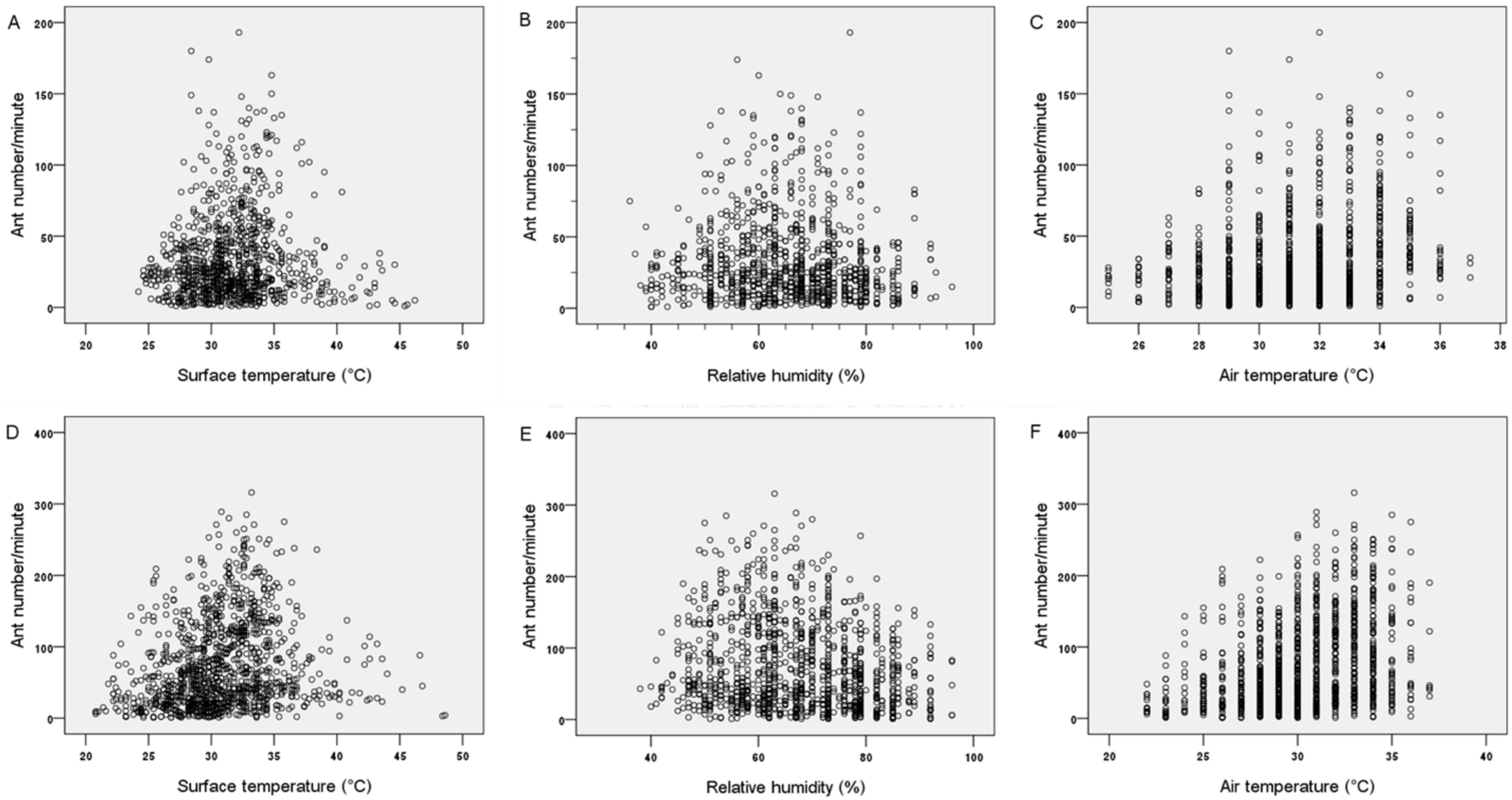

Figure 28 The scatter plots of raw data of the returning ant numbers/minute during December 2014 – November 2015 against surface temperature, relative humidity and air temperature in regular ant activity (A,B and C) and ant activity with bait (D,E and F).

Interestingly, during 25 – 27 January 2016, the strong high air pressure from People's Republic of China extended to upper Socialist Republic of Vietnam and the upper region of Thailand as well. The weather conditions in Bangkok was predicted to vary first with thundershowers and strong wind before sharp cold spell expected with decreasing temperature by 6–10°C and the minimum temperature would be 16–18°C (prediction from the Thai Meteorological Department). Therefore, on 26 January 2015, the foraging activity was conducted again on three ant colonies (SCS, SCL and CHL) to see how the low temperature affected Singapore ant activity (Figure 29).

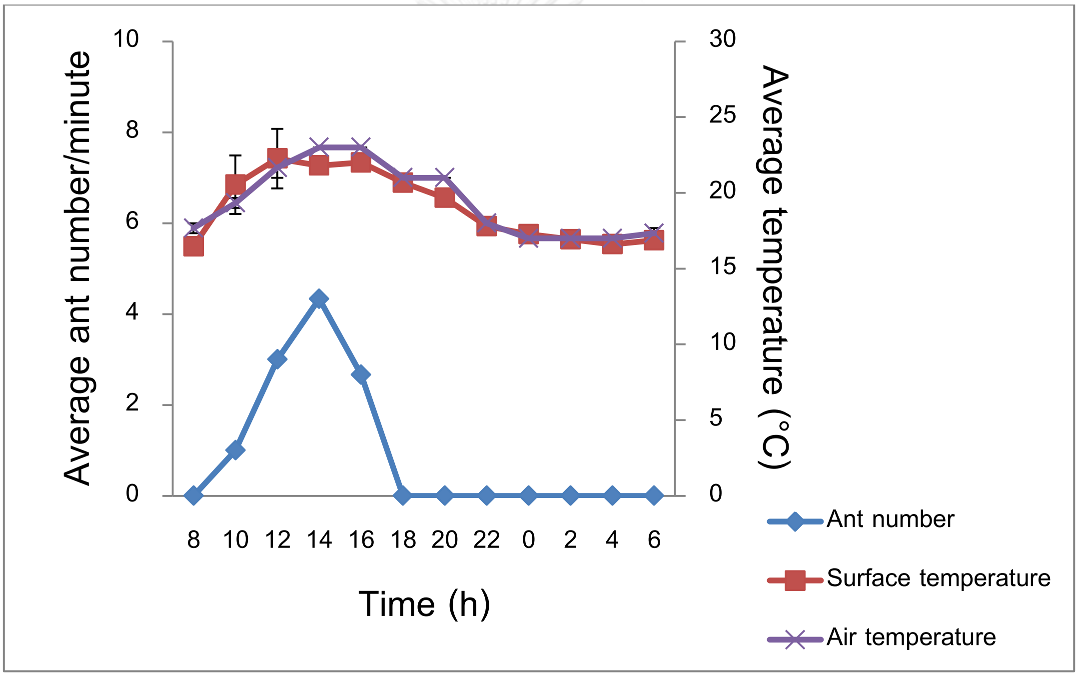

Figure 29 The average foraging activity of the three Singapore ant colonies (SCS, SCL and CHL) with average temperature on-one day period (26 January 2016)

During the rare cold period in Bangkok, the result revealed that the ants never foraged when the surface temperature or ambient temperature dropped down below 20°C. During that period, the ant activity was very low, with only few ants went outside their colonies. On the other hand, the unusually high surface temperature which exceeded 50°C also limited the ants to forage despite the bait was present (Figure 30).

Figure 30 There was no ant foraging to the bait (red circle) because of the unusually high surface temperature in afternoon.

4.3.3 Foraging range

The foraging range data did not fall on parametric criteria, so the data were applied on non-parametric statistics. The result in this study showed the significant difference in foraging ranges between dry and wet seasons (Mann-Whitney U test: U = 1654, n_1 = 50, n_2 = 108, *p-value* < 0.001). The average foraging ranges (± SE) of dry and wet seasons were 1.33 ± 0.16 m and 0.76 ± 0.05 m, respectively. This indicated that the foraging range of Singapore ant in dry season was farther than in wet season.

It seemed that during the wet season, food was easier to find when compared to dry season. During the wet period, there were a lot of prey or carcasses such as miniature awlsnail, *Subulina octona*, and black – spined toad, *Bufo melanostictus* (Figure 38).

Figure 31 Singapore ants fed on a miniature awlsnail and a carcass of black – spined toad (in red circle) during the wet season.

4.3.4 General foraging behavior observation

In the foraging activity study, the ant colonies were observed for 12 months. There were other observed behaviors as reported below.

4.3.4.1 Ants' communication for food location

When the first worker ant (patroller) had detected and tasted the bait, it immediately returned to the trail and then communicated to the other workers via antenna contact. However, some observations revealed that, sometimes, the patroller vigorously contacted other worker ants' bodies besides antenna contact. After the other worker ants were activated by the patroller, the workers simultaneously formed a new trail to the food where patroller had previously found. The communication for food location quickly took place because the ants activated by patroller could rouse other ants like a cascade reaction. Moreover, the leader ant which led the worker ants to the food source was not necessary to be the patroller.

4.3.4.2 Closing the nest entrance

This behavior could be observed before the sunset. The ants always tended to use materials around the nest entrance to close or cover it. From the observations, these materials were small pieces of leaves, small seeds and grits. Not all ant colonies performed this behavior because some colonies were located in crevice of the building or cement structure, so it may be difficult to cover all the nest entrance along the crevice. The colonies which expressed this behavior were likely to settle in the soil or holes of the cement structure (Figure 31).

Figure 32 The nest entrance covered with a grit (red circle).

4.3.4.3 Relationship with other species

In the natural habitats, many organisms live together in the ecosystem and inevitably exhibit some relationships or interactions to other organisms. Likewise, from these observations, the relationships between Singapore ant and other organisms were recorded.

Once the bait was placed, the other ant species might arrive at the bait before Singapore ant, but none of the other ant species could compete Singapore ant for the food resource. *Paratrechina longicornis* (Figure 32), longhorn crazy ant, and *Pheidole parva* (Figure 33-34) were the ant species which retreated when Singapore ant presented at the same food source. However, there was one exception for *Tapinoma melanocephalum* (Figure 35), the ghost ant, which could seize the bait from the Singapore ant using scent. The effect of scent produced by ghost ant made Singapore ant stunned but not deadly (Figure 36). Although *Pheidole parva* retreated when Singapore ant appeared, *P. parva* attacked Singapore ant when passed its nest entrance. The assault of *P. parva* was dragging an enemy (Singapore ant) into its nest and then sieged the hapless enemy.

Figure *33 Paratrechina longicornis* worker

Figure 34 *Pheidole parva* worker (Minor)

Figure 35 *Pheidole parva* worker (Major)

Figure 36 *Tapinoma melanocephalum* worker

Figure 37 *Tapinoma melanocephalum* (in red circle) invaded a Singapore ant's nest.

Another observed interaction besides competition was parasitism. This type of relationship occurred between Singapore ant and kleptoparasitic (parasitism by theft) silverfish, Order Thysanura. This silverfish was usually found during nighttime by walking out of ant nest in ant trail (Figure 37).

Figure 38 A silverfish (in red circle) walked among worker ants.

4.4 Discussion

4.4.1 Daily foraging activity

In this study, maximum foraging activity of *T. destructor* occurred from 4 pm to 6 pm (Figure 14). This peak activity occurred similar to peak activity of *Monomorium pharaonis* which occurred during evening hours (Mallis, 1997). Different ant species displayed the different peak activities, for instance, peak activity of *Solenopsis geminata* occurred around midnight to early morning (11 pm to 3 am) (Norasmah et al., 2006).

The result in this study showed that the sunrise time and sunset time did not influence Singapore ant activity (Figure 15). In some ant species, the sunset time affected the onset of foraging such as in *Myrmecia pyriformis* and *Odontomachus chelifer* (Jayatilaka et al., 2011; Narendra et al., 2010; Raimundo et al., 2009). For example, the onset activity of *M. pyriformis* was influenced by light intensity at sunset to avoid both competition and predators, and using of polarized light for orientation which is simplest when sunset (Narendra et al., 2010). Light also has impacts on ant foraging activity, for example, Subterranean *Eciton* ants exhibited that light was a determining factor for emergent time (Vowles, 1995). Several studies reported that surface temperature influenced the starting time of ants' foraging activity. For instance, the emergent time of *Myrmecia croslandi* depended on surface temperature during sunrise time to avoid both high and low surface temperatures (Jayatilaka et al., 2011). *Pogonomyrmex occidentalis* also showed the correlation between the activity onset and surface temperature which the minimum temperature was more correlated than the maximum temperature (Cole et al., 2010). Temperature is also the main factor that control colony activity and metabolism (Porter and Tschinkel, 1993).

Singapore ant seemed to forage throughout the day but the activity during day time was much greater than nighttime (Figure 14). Interestingly, during nighttime in dry season (Figure 15A, 15C), the Singapore ant activities, both regular activity and activity with bait, were not very much different. This may be the results of low humidity and nighttime which cooperatively decreased the influence of bait to the ants. It has been known for a long time that Singapore ant is one of the most successful invasive ants. There have been many mentioned factors influencing the achievement of invasive species. One of these factors is behavioral flexibility (Wright et al., 2010). Singapore ant is one of the opportunistic creatures which forages throughout the day. The reasons why Singapore ants tend to forage throughout the day may be the lack of competitors in urban environment compared to the natural environment. Also there is a hypothesis that Singapore ant in their native range (not in the urban environment) could be a diurnal ant but when it moves into human community, it then adapted its behavior to maximize resource exploitation. In contrast, some ants forage only during daytime while other ants forage only during nighttime. For example, the two sympatric *Myrmecia* ants which showed the different time of foraging. *M. croslandi* is a diurnal ant where as *M. pyriformis* is a nocturnal ant (Jayatilaka et al., 2011).

In this study, Singapore ant activity with bait was much higher than the activity without bait as well as the results from paired hours between activity without bait and activity with bait (Figure 16). *T. destructor* adopted a special strategy in food collection, mass recruitment, which the successful worker lays trail pheromone attracting a large number of ants to food location. This strategy signals workers to forage in the overwhelming number (Lach et al., 2010). However, during the early afternoon in the study period, the number of foraging Singapore ants affected by trail pheromone declined (Figure 16). This may be because of high temperature and low humidity during the afternoon. This was concordant with the study of foraging activity of Argentine ant, *Linepithema humile*, which its activity was limited by the high temperature and low humidity (Abril et al., 2007).

In this study, the Singapore ant activity in wet season was significantly higher than the activity in dry season (Figure 18), and the relative humidity in wet season also was significantly higher than in dry season (Table 3). A lot of researches also reported that the activity pattern of many ant species in wet season was higher than in dry season. In *Odontomachus chelifer*, the foraging activity in wet season was distinguishably higher than in dry season because the greater number of prey and larvae (Raimundo et al., 2009). The study of humidity preference of *Linepithema humile* and *Iridomyrmex* sp. showed that, by 24 hours, the most numbers of both ant species preferred the highest humidity containers and the high survival rates of this two species also occurred in the highest humidity containers (Walters and Mackay, 2003). Moreover, there is another explanation of the relation between humidity and foraging activity of harvester ant, *Pogonomyrmex barbatus*. It might be because humidity has impacts on the sense of chemical cue influencing the foraging activity (Gordon et al., 2013). The results from paired hours between regular ant activity without bait and ant activity with bait in dry season showed the differences in ant mean numbers only during daytime. This may be because of the low humidity, low temperature and strong wind during nighttime. In contrast, there were significant differences in ant mean numbers between paired hours in wet season both daytime and nighttime. This may be the result of high humidity in wet season throughout the day.

The foraging patterns of the three Singapore ant colonies in this study were distinct (Figure 20-22). This distinction is a variation in foraging pattern that can be normally occurred as a result of many factors which shape each colony to have individual or unique personality. The factors that shape colony personality are the average personality of workers, the distribution of worker personalities, the local environment and colony genetics (Jandt et al., 2014; Pinter-Wollman, 2012).

4.4.2 Correlation and relationship between foraging activity and environmental factors

Although the relationship result from quasi-Poisson showed that only surface temperature and air temperature significant influenced ant activity, relative humidity was not significant (*p-value* = 0.0536) (Table 4). In this study, relative humidity did not show the very clear effect on Singapore ant activity. It may be because the year round average relative humidity in this study was relatively high at around 60% (data from field collection) and around 73% (data from the Thai Meteorological Department station in Bangkok during 2014 to 2015). However, a number studies found that many ant species' foraging activities were affected by physical factors (Abril et al., 2007; Bernstein, 1979; Cerda et al., 1998; Chong and Lee, 2006; Narendra et al., 2010; Raimundo et al., 2009).

In this study, in the ant activity with bait during an afternoon, the surface temperature exceeded 50°C (50-60°C) and no ant walked to the plastic station located outdoors (Figure 30). However, there are some ants that are thermophile such as *Forelius nigriventris*. The occurrence of *F. nigriventris* was postively correlated with surface temperature and its peak activity also happened during high surface temperature (Bestelmeyer, 1997).

In general, the high air temperature was a limiting factor to ant foraging activity (Abril et al., 2007; Chong and Lee, 2006). Not only high air temperature affects ant activity but low air temperature also influences ant activity. In this study during the cold period in January 2015, the Singapore ant never foraged when the surface temperature or air temperature dropped lower than 20°C (Figure 29). To sum up, the temperature at high and low ends both limit ant activity depending on ant species as shown in many previous studies (Cerda et al., 1998; Jayatilaka et al., 2011; Kay and Whitford, 1978).

From this study, the average surface temperature and air temperature between dry and wet seasons were not much different. In contrast, the average relative humidity between wet and dry seasons was different (Table 3). The relative humidity may be one of the important physical factors that responsible to the activity change in different seasons (Figure 19). This result was consistent with previous reports that ant activities responded positively to the relative humidity (Chong and Lee, 2006; Chong and Lee, 2009; Talbot, 1943). Furthermore, the higher ant activity in wet season than in dry season may be the results of avoiding the desiccation risk and there was more food during wet period (Kaspari and Weiser, 2000).

Size matters to desiccation in ants. The larger ants tend to be more resistant to water loss (Hood and Tschinkel, 1990). Singapore ant is one of small sized ants which its total length ranging from 1.8 to 3.5 mm (Bolton, 1987). This makes Singapore ant easily to desiccate in the unfavored environment. However, this study revealed that Singapore ants were more tolerant to the harsh environmental conditions when the bait was present (Table 5, Figure 28). According to optimal foraging theory, animals should maximize energy gain per unit of time. It is likely to be explain via the profit that animals require as shown in simple equation below:

$$Profitability\ of\ prey\ = \frac{Energy\ per\ prey\ item - Costs\ to\ acquire\ prey}{Time\ taken\ to\ acquire\ prey\ item}$$

(Sinervo, 2013)

The result from species response curve (Table 5) was also consistant with optimal foraging theory. Environmental tolerances (ET) of Singapore ants were wider when bait was present and the ants were more tolerant to hotter surface temperature and lower relative humidity. Singapore ants may choose whether to collect the bait among relatively low and high temperature conditions. The reasons providing the ants to forage in unfavored condition may be explained by the mentioned equation above. The ants may be sure that they are profitable to tradeoff. Costs to acquire prey in this study has mentioned only the physical constraints (not include predation risk) which did not reach the lethal point. At the same time, the time spent to acquire food was not too long because the bait location was set around the often used ant trail. Thus, it is not surprising why Singapore ants accepted to forage in the wider physical range when bait was presented.

4.4.3 Foraging range

The result indicated that foraging range of Singapore ant in dry season was greater than in wet season. During wet period, there was abundant food supply such as prey and carcass items, so it is no need for ants to forage for a long distance. In contrast, in dry season there were not as abundant food supplies as in wet season. Therefore, ant workers had to travel for longer distance.

However, some studies showed contradictory result with this study. They were reported that the foraging range of *Gnamptogenys moelleri* and *Pachycondyla striata* in rainy season was larger in size than in dry season because there were more ant broods and prey items during that period (Cogni and Oliveira, 2004; Medeiros and Oliveira, 2009). The difference between previous studies and current study may be due to different habitat types. The former studies were conducted in forest areas while this study was conducted in urban area. Therefore, different habitat types may lead to the differences in food availability, food distribution and the number of competitors which may influence ant foraing behavior including foraging range.

4.4.4 General observation

When a patroller found the food resource, it took time for a while to taste food and then walked back to the main trail/nest entrance with somewhat tortuous straight line. This path was not the same line leading to the food source. Ants can precisely move to destination using two navigation system, sun compass and, in some, polarized light (Wehne and Muller, 2006). For Singapore ants, traveling back to main trail/nest entrance without using the same direction may adopt either system to navigate. Once the patroller reached other workers, antenna contact or body contact occurred, then the new trail was formed to the food resource. The newly tortuous path was formed during early formation and later overlapped with the trail pheromone left by the patroller which lead to the resource location. Thus, Singapore ant primarily relied on smelling sense to navigate to food source.

Closing the nest entrance is not a novel issue. Workers of some ant species have specialized head to block nest entrance (Lach et al., 2010). This behavior called “phragmosis” which may aim to protect the nest from predators or unsuitable environment. However, Singapore ants do not have such special organ, so workers may have to find some materials to cover the nest entrance.

Due to the fact that Singapore ant adopts mass recruitment strategy for forging, it is difficult for many other ant species to compete with Singapore ants for the same resources. However, *Tapinoma melanocephalum* or ghost ant has special chemical weapons to outcompete other ants and these chemical are 6-methyl-5-hepten-2-one and actinidine which produce the foul odor. The former is released as alarming signal while the latter is used as repellent. The result of these compounds was consistent to this study that the compounds caused the hostile ant (*Solenopsis geminata*) staggered and prostrated (Tomalski et al., 1987).

Another observed relationship is parasitism which occurred between Singapore ant and silverfish. This primitive insect lives in ant colony and steal ant food for consumption. Silverfish hide themselves from ant workers by rubbing with ant callows to gain cuticular hydrocarbons (CHCs) which pose as marking for nestmate recognition. These CHCs degrade over time, so silverfish have to continually polish with the callows. Otherwise, silverfish will be attacked by the ant workers (von Beeren et al., 2011).

4.5 Conclusion

The maximum foraging activity of Singapore ant occurred during late afternoon to early evening (around 4 pm to 6 pm). This ant usually foraged throughout the day, and the ant activity during daytime was much greater than ant activity during nighttime. The ant activity with bait was greater than regular ant activity (without bait). This indicated that the presence of bait (or food source) definitely influenced the ant activity. Sunrise and sunset times also did not have any influence on emergent time or cessation time of this ant species.

Season also influenced Singapore ant activity in which the ant activity in wet season was greater than the activity in dry season regardless of the presence of bait. All year round, each Singapore ant colony exhibited unique foraging pattern, and yet, they shared the elemental similarity. The result from quasi-Poisson regression pointed out that surface temperature and air temperature had effects on the ant activity while the effects of relative humidity was not clear. For species response curve, the results showed that the presence of bait caused the Singapore ants were more tolerant to the harsh environmental conditions.

The ant communication for food location occurred among ant workers via body or antenna contact and trail pheromone laid by the ant worker that firstly detected the resource location. This communication could rapidly happened through only one successful worker by amplification signal to the others.

Singapore ants closed their nest entrance if the entrances were holes on the ground. These holes were covered with small seeds or grits before the sunset. In this study when there were competitions for food between ant species, Singapore ant can outcompete most ant species except ghost ant, *Tapinoma melanocephalum*, which used chemical scent to subdue Singapore ants. Singapore ants also inhabited with silverfish which stole the ants' food in the colony. In case of foraging range, Singapore ants clearly showed farther foraging distance during dry season than wet season.

CHAPTER V

Food preference and ant abundance

5.1 Introduction

Ants are holometabolous insects which include four developmental stages: egg, larval, pupal and adult stages. Each stage needs different kinds of food for different purposes, for instance, the larvae need nutrients for growth while the adults need food to maintain general body functions (Lach et al., 2010). There are both intrinsic and extrinsic factors determining ant food preference.

The good understanding of food preference in urban pest ants would provide to maximize the effectiveness of ant control and management program using baits. In 2007, Eow and Lee reported that *Trichomyrmex destructor* preferred carbohydrate over protein and lipid. However, the study was conducted under laboratory condition and not covered all seasons. Thus, the objectives of this study were to examine whether Singapore ant, *T. destructor*, exhibited the different food preferences according to the different seasons and to look for the particular bait that ants usually detected first. The ant abundance data were also recorded to roughly estimate the ant population throughout a year.

5.2 Materials and Methods

5.2.1 Food preference

Food preference was carried out on four ant colonies (TNB, CML, CHB and ENG), three times a month (after being conducted the study of ant abundance) from December 2014 to November 2015. The baits used in this study are based on different food (protein, carbohydrate and lipid) and its properties (solid and liquefied substances). The 20% (w/v) of sucrose solution, 20% (w/v) of sucrose solution agar (agar: water = 0.1 g: 10 ml), pork liver (piece), ground pork liver (paste), pork fat (piece) and lard were used as baits. Sucrose solution and sucrose agar represented carbohydrate source, pork liver and ground pork liver represented protein source and pork fat and lard represented lipid source. Sucrose solution, ground pork liver and lard were represented as liquefied bait form, and sucrose agar, pork liver and pork fat were represented as solid bait form. The baits weighed around 0.5 to 0.6 g were placed randomly every replicate on plastic pads (1.9 cm x 12 cm) and left for 15 minutes along the ant trail (Figure 39). After 15 minutes, each plastic pad with bait was photographed (by Olympus OM-D E-M1) and the number of ants on each pad from the captured images was counted on a computer.

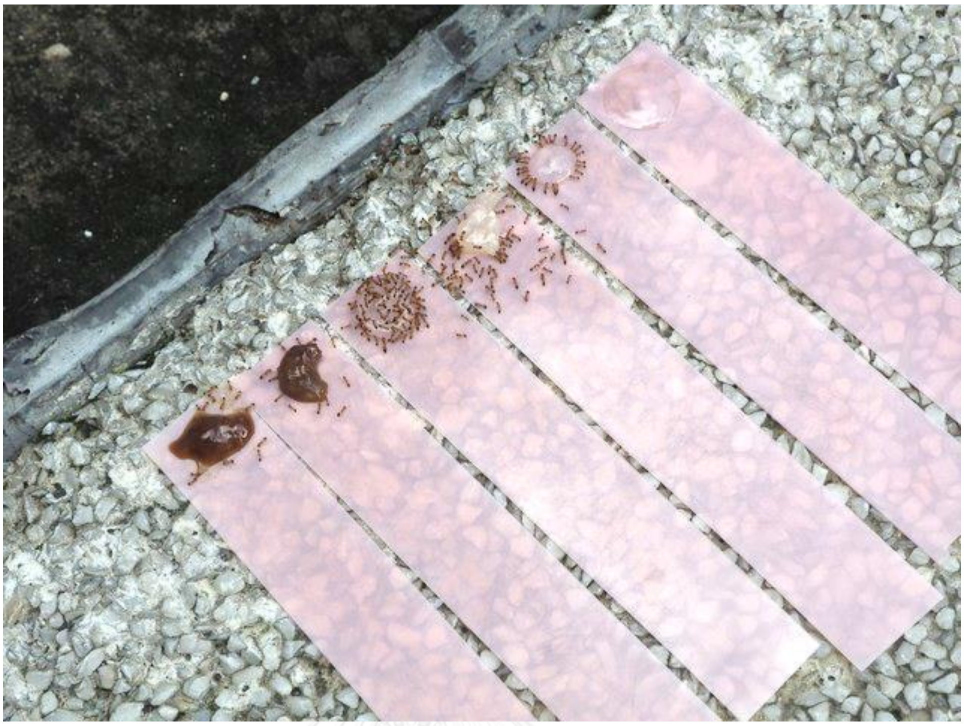

Figure 39 The six bait choices were laid on plastic pads along the trail in food preference study. This picture showed the six bait choices, ground pork liver, a piece of pork liver, sucrose agar, a piece of pork fat, sucrose syrup and lard (from left to right).

The orders of baits detected by Singapore ants were also recorded with score ranged from 1 to 6. The 1st, 2nd, 3rd, 4th, 5th and 6th bait detected were scored as 1, 2, 3, 4, 5 and 6 points, respectively. In this study, the firstly detected bait was determined by the first worker ant which its mandible touched the particular bait. Then, the first detection frequencies of each bait type were calculated.

5.2.2 Ant abundance

Bait was used to study ant abundance (Agosti et al., 2000). Mixture of crushed cookie and crushed peanut (1:1 w/w) was used to study ant abundance of the four ant colonies (TNB, ENG, CHB and CML) for 12 consecutive months from December 2014 to November 2015. In each month, ant abundance had been conducted before being conducted the study of food preference. Four plastic pads (8.0 x 8.0 cm) were placed around a nest entrance of the studied ants. A half teaspoonful of bait mixture was placed on each plastic pad at about the middle point of the pad edge near the nest entrance (Figure 40). The bait was left for 10 minutes, then each plastic pad with bait was picked and put into a plastic bag, and frozen in a refrigerator before counting the total numbers of ants.

Figure 40 The crushed cookie and crushed peanut (1:1 w/w) was put on plastic pads which placed around the ant nest entrance (red circle) for ant abundance study.

5.2.3 Data analysis

To discriminate the different seasons (wet and dry seasons), the criterion of Walter et al. (1975) was also adopted as in Chapter IV and the result was that the dry season was in December 2014, January 2015 to February 2015 and November 2015, while the wet season was from March 2015 to October 2015.

Food preference data were checked for normality and equal variances before conducting further analyses. If the data did not reach the assumptions for parametric analyses, the non-parametric analyses were then applied. Average ant number/trap of six bait choices were compared whether there were any significant differences among the different baits in different time periods, including dry season and wet season. If there were any significant differences among six bait choices, average ant number/trap of each bait type was paired and then compared to examine the significant difference between each bait pair. Further, the average ant number/trap of both solid bait and liquefied bait were compared.

Chi-square test was utilized to examine the difference between expected frequencies of firstly detected bait and observed frequency of firstly detected bait. In addition, the sums of given score were calculated for ranking the detected baits; and the lowest sum score bait implied that the bait was usually firstly sensed by Singapore ant and vice versa. All statistical tests in this chapter were conducted using the SPSS software (version 17.0).

5.3 Results

5.3.1 Food preference

Kolmogorov-Smirnov test was analyzed to examine whether the food preference data were normal distribution. However, these data sets did not reach parametric assumption. Then, Kruskal-Wallis test was used to analyze whether there were any significant differences among average ant number/trap of six bait types in different time periods, including both in dry season and wet season.

The results showed that there were significant differences of ant numbers among six bait choices (Kruskal-Wallis: $X^2 = 225.911$, df = 5, *p-value* < 0.001), and there were significant differences both in dry season (Kruskal-Wallis: $X^2 = 56.375$, df = 5, *p-value* < 0.001) and wet season (Kruskal-Wallis: $X^2 = 197.129$, df = 5, *p-value* < 0.001).

After performing Kruskal-Wallis test, Mann–Whitney U test was applied to find a significant difference between two pair bait choices. The mean numbers of ant/trap (± SE) of six bait choices in one year round is shown in Table 6. The result from Man-Whitney U test revealed that the most preferable baits were sucrose agar and pork liver, while lard was the least preferable bait (Figure 41).

Table 6 Means (± SE) of ant number/trap found on a plastic pad of each bait type in one year round

Bait	Mean ± SE (individual/trap)
Sucrose syrup	22.56 ± 1.86^{b}
Sucrose agar	40.74 ± 2.92^{a}
Pork liver (piece)	38.71 ± 3.33^{a}
Ground pork liver (paste)	18.26 ± 1.97^{b}
Pork fat (piece)	24.74 ± 2.66^{b}
Lard	2.40 ± 0.35^{c}

Note: Different letters (a, b and c) indicate statistical significances of mean numbers (Mann-Whitney U test: $p < 0.05$).

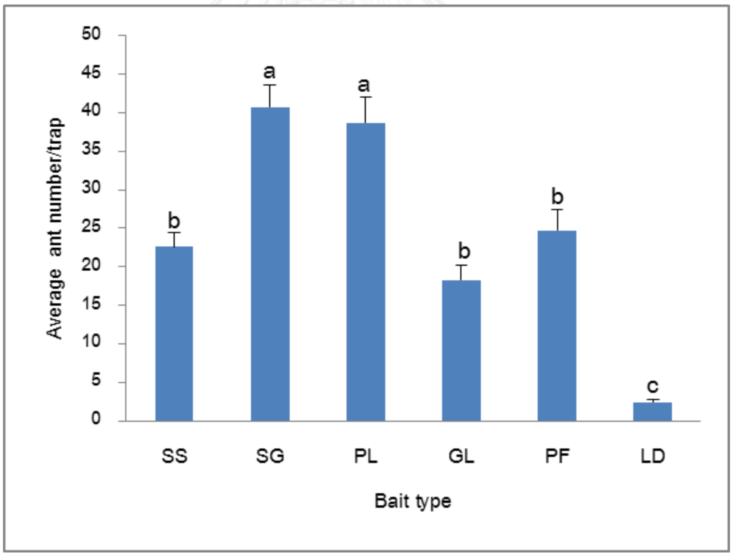

Figure 41 Means (± SE) of ant number/trap of each bait type in one year. (SS = sucrose solution, SG = sucrose agar, PL = pork liver, GL = ground pork liver, PF = pork fat and LD = lard)

Note: The different letters (a, b and c) indicate statistically differences of means of ant number.

Moreover, when the results were examined in the different seasons (wet season and dry season), the data showed the dissimilarity in food preference across different seasons. The mean numbers of ant/trap (± SE) of six bait choices both in dry season and wet season were shown in Table 7 and Table 8, respectively. The result from Man-Whitney U test showed that during the dry season, pork liver was the most preferable by Singapore ant (Figure 42), whereas during the wet season sucrose agar was the most favored among other baits (Figure 43).

Table 7 Means (± SE) of ant number/trap found on a plastic pad of each bait type in dry season (December 2014, January 2015 - February 2015 and November 2015)

Bait	Mean ± SE (individual/trap)
Sucrose syrup	15.31 ± 3.28^{b}
Sucrose agar	22.06 ± 4.23^{b}
Pork liver (piece)	36.46 ± 5.76^{a}
Ground pork liver (paste)	14.56 ± 3.46^{b}
Pork fat (piece)	19.79 ± 3.44^{ab}
Lard	1.88 ± 0.57^{c}

Note: Different letters (a, b and c) indicate statistical significances of mean numbers (Mann-Whitney U test: $p < 0.05$).

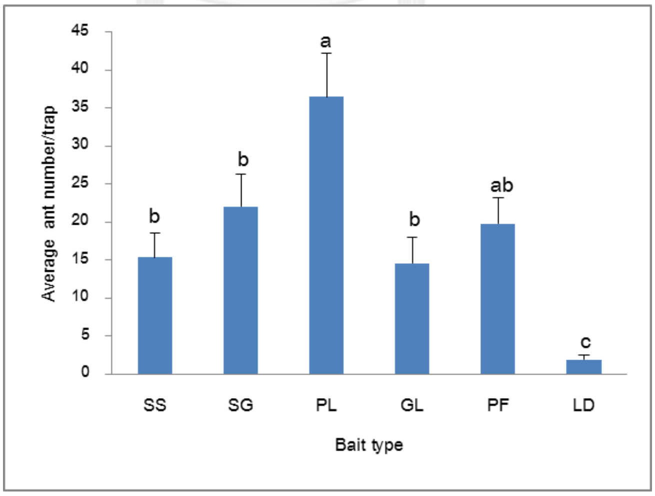

Figure 42 Mean (± SE) of ant number/trap of each bait type during dry season. (SS = sucrose solution, SG = sucrose agar, PL = pork liver, GL = ground pork liver, PF = pork fat and LD = lard)

Note: The different letters (a, b and c) indicate statistically differences of means of ant number.

Table 8 Means (± SE) of ant number/trap found on a plastic pad of each bait type in wet season (March 2015 - October 2015).

Bait	Mean ± SE (individual/trap)
Sucrose syrup	26.19 ± 2.17^{b}
Sucrose agar	50.08 ± 3.47^{a}
Pork liver (piece)	39.83 ± 4.09^{b}
Ground pork liver (paste)	20.10 ± 2.39^{b}
Pork fat (piece)	27.22 ± 3.59^{b}
Lard	2.67 ± 0.44^{c}

Note: Different letters (a, b and c) indicate statistical significances of mean numbers (Mann-Whitney U test: $p < 0.05$).

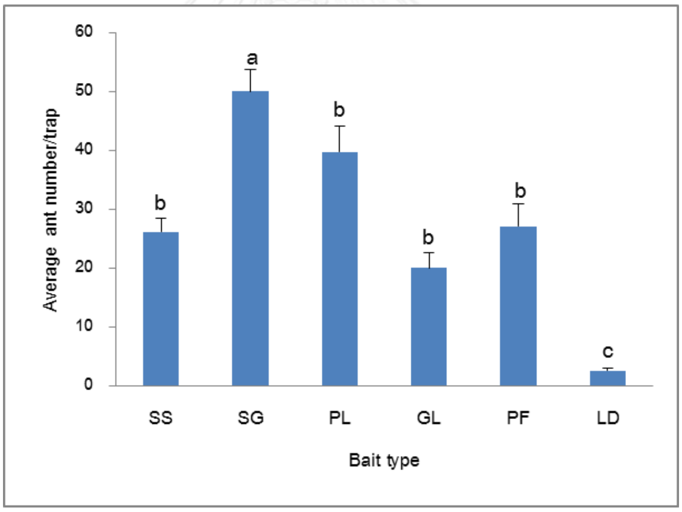

Figure 43 Mean (± SE) of ant number/trap of each bait type during wet season. (SS = sucrose solution, SG = sucrose agar, PL = pork liver, GL = ground pork liver, PF = pork fat and LD = lard)

Note: The different letters (a, b and c) indicate statistically differences of means of ant number.

To roughly assess the effect of bait formulations on ant food preference, the two bait forms (solid and liquefied substances) were employed. The mean numbers (± SE) of ant/trap in solid and liquefied baits in one year, and in the dry season and the wet season are shown in Table 9. The results from Man-Whitney U test presented the consistence in solid bait preference over liquefied bait in both dry and wet seasons (Mann-Whitney U test: U = 6771.5, n_1 = 144, n_2 = 144, *p-value* < 0.001; Mann-Whitney U test: U = 23423.0, n_1 = 288, n_2 = 288, *p-value* < 0.001, respectively) and also in all year round (Mann-Whitney U test: U = 55974.0, n_1 = 432, n_2 = 432, *p-value* < 0.001) (Figure 44).

Table 9 Means (± SE) of ant number/trap found on plastic pads of solid and liquefied baits in one year round, dry season and wet season.

	Mean ± SE (individual/trap)	
	Solid baits	Liquefied baits
One year	34.73 ± 1.75^a	14.41 ± 1.00^b
Dry season	26.10 ± 2.70^a	10.58 ± 1.67^b
Wet season	$39.05 \pm 2.21a^a$	16.32 ± 1.23^b

Note: Different letters in the same row indicate statistical significances of mean numbers (Mann-Whitney U test: $p < 0.05$).

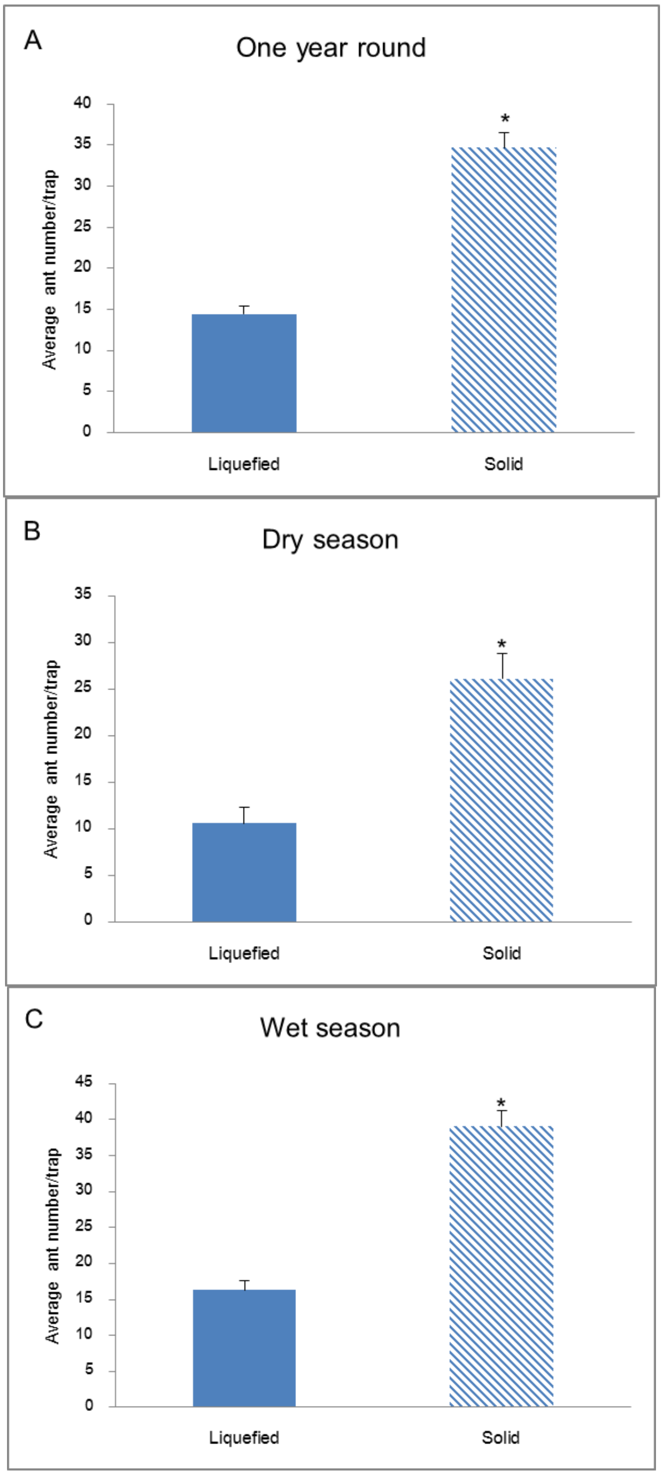

Figure 44 Mean (± SE) of ant number/trap of liquefied and solid form baits during A) one year round, B) dry season and C) wet season.

Note: The asterisk indicates the significant difference between mean ant numbers in liquefied and solid baits (Mann-Whitney U test: $p < 0.05$).

When a piece of solid bait had been left too long, the worker ants bit and cut the bait into small pellets as shown in Figure 45.

Figure 45 Sucrose agar was cut into small pellets (in the red circle) by Singapore ants in the case when bait was placed for a long time.

There was a difference between the observed frequency and expected frequency of the first detection of each bait by the ants (Chi square test: $X^2 = 124.72$, df = 5, *p-value* < 0.001). Therefore, the statistic result reiterated that the first detected bait by Singapore ants was not occurred by haphazard. This means that six bait choices did not show equal attraction potential.

The summary of the first detected bait frequency and the summary score with rank of each bait type were shown in Table 10. The given score showed that pork liver (piece) had the lowest score while lard had the highest score. These mean pork liver was usually first detected by Singapore ants among six bait choices and lard was usually last detected.

Table 10 Firstly detected bait frequency of Singapore ants and summary score with rank of each bait type

Bait type	First detection frequency (time)	Summary score (point)	Rank
Pork liver (piece)	53	196	1
ground pork liver (paste)	31	232	2
20% (w/v) of sucrose solution agar	12	355	3
Pork fat (piece)	7	357	4
20% (w/v) of sucrose solution	0	478	5
Lard	1	566	6

5.3.2 Ant abundance

The ant numbers of four ant colonies trapped for 12 consecutive months were plotted along with the average ant number/colony (Figure 46). The graphs showed the fluctuation of ant numbers among four ant colonies. However, the average ant number/colony indicated the ant abundance during dry season (around from December 2014 to March 2015) was slightly lower than other periods of the year.

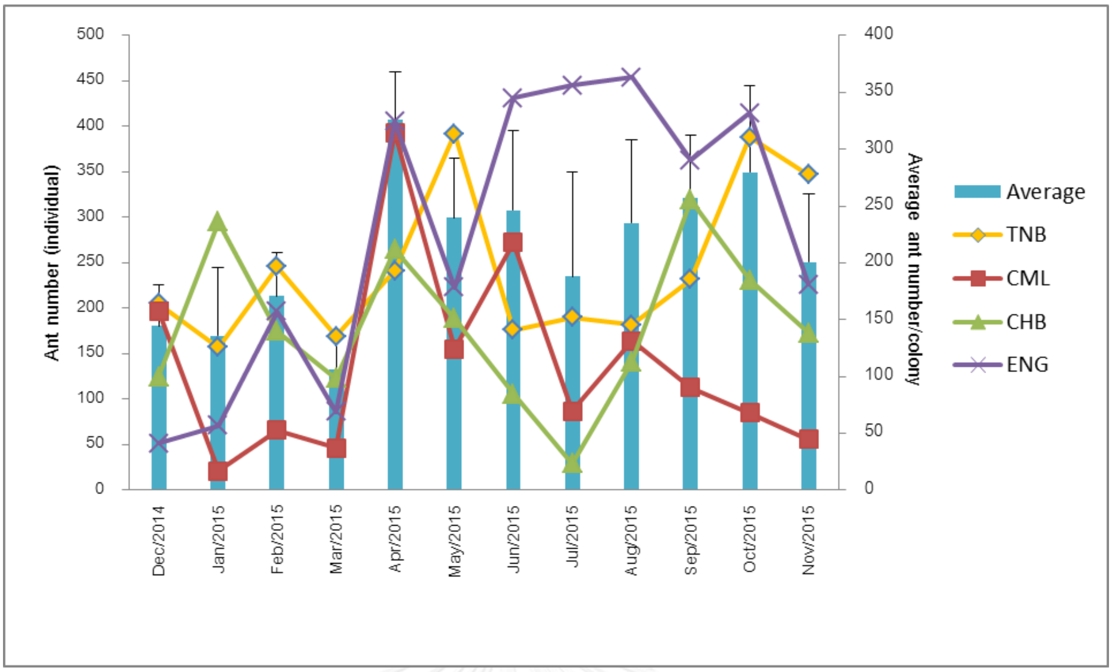

Figure 46 The line graphs of four Singapore ant colonies' abundance (TNB, CML, CHB and ENG) were plotted along with average ant number/colony (bar chart) in one year.

5.4 Discussion

5.4.1 Food preference

In this study, the result of food preference of Singapore ant for 12 months substantiated that the two most preferred baits were sucrose agar and pork liver (piece). This may be because ant workers needed protein to rear queens and larvae living in their nest, and carbohydrate for themselves. Carbohydrate is not only a source of energy for workers and enhances larval development but also is utilized to generate lipid reserves, whereas protein is a major source for larval growth (Cook et al., 2011; Dussutour and Simpson, 2009; Mashaly et al., 2013).

The results still indicated that Singapore ants were rarely attracted by lard when compared to other bait types. However, this does not mean that ants are not attracted by oily baits. The previous studies showed that *Solenopsis nitens* and *Solenopsis invicta* all attracted by oily based baits (Glunn et al., 1981; Sengupta et al., 2010).

The result in this study showed the difference of bait preference according to the different seasons. It is possible that ants differently respond in food preference due to the different seasons. For example, *Solenopsis invicta* exhibited the difference in food preference which during the colder period the ants preferred carbohydrate while protein was perferred during the warmer period. These indicated that in colder period of the year, the natural sugar source is scarce, so the ants are preferred in carbohydrate. On the other hand, during the warmer period, colony grows so that protein food is more desirable (Stein et al., 1990). Moreover, Cook et al., 2011 reported that ants collecting different food in different seasons and seasons played an important role to ant nutrition regulation. In this study, the difference in food preference in different seasons may be the result from the difference in nutritional demands, which may reflect in different colony's cycle such as the period of reproduction.

Besides nutritional demands, food availability is another important factor to determine colony preference. The study of food preference of Yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island showed the consistent result with this study that protein was more preferable in dry season while carbohydrate was more preferable in wet season. This was explained by the shortage of protein source (small invertebrates) in dry season and carbohydrate source (honeydew) from scale insects in wet season (Abbott et al., 2014). However, the life cycle of Singapore ant in year round still needs further investigations.

Ants were determined as collectors or hoarders of excessive food (Gayahan and Tschinkel, 2008). In this study, ant workers preferred solid bait over liquefied bait and transported food in solid form into colony with various sizes of food. The food sizes ranged from tiny pellet extracted from sucrose agar to small fragments of cookie and peanut. This conveyance of solid food into colony indicated that Singapore ant showed hoarding behavior for reserving food for future use or waiting for digestion by larvae. The hoarding of solid food was more advantageous than hoarding liquid food because it was easier to handle. Otherwise, the ants should have special morphology such as in Honeypot ants, *Myrmecocystus* spp., to retain liquid food. However, it is well known that adult ants cannot take in solid food because the small diameter of ant petioles (Cassill et al., 2005). Moreover, adult ants still have special organs to prevent large solid particles to pass to their digestive system such as in red imported fire ants, *Solenopsis invicta*, and *Camponotus americanus* which show infrabaccal pocket and buccal tube as filter system. Once the pocket is full, the particles will be packed and expelled as a pellet (Michael Glancey et al., 1981). By the way, why do ants still carry pieces of solid food back to their nests despite they cannot even to eat? The simple answer of this question involves ant larvae. Ant larvae play a crucial role in colony as a digestive caste (Cassill et al., 2005), but not every ant instar can do this task. Only the fourth instar (mature larvae) of ants can process solid food form to liquid form, so the earlier instars and adult ants are characterized as liquid feeders (Michael Glancey et al., 1981).

Ant larvae have specialized organ located on ventral region to help digestion of solid food. However, not all larval ant species have specialized organ such as *Crematogaster laeviuscula* and *Iridomyrmex pruinosus* (now is *Forelius pruinosus*). The simplest structure of holding food was found in Myrmicinae such as *Monomorium pharaonis* and *Solenopsis molesta*. Ant from the same subfamily (Myrmicinae) such as *S. invicta* and *Trachymyrmex septentrionalis*, showed the well developed stucture with arranged hair and hairless area acted as receptacle for food particle and was called “food basket”. The more developed structure called “food platter” was found in most Ponerinae and some Mrymicinae such as *Pogonomyrmex barbatus*, whereas the most advanced structure called “food pocket” was found in Subfamily Pseudomyrmecinae and Tribe Camponotini. This showed that the trait evolved independently in many ant taxa (Petralia and Vinson, 1979).

In *Pheidole spadonia*, there were several steps that showed the cooperation in solid food digestion between workers and larvae. Firstly, after prey items were delivered into nest, the adult ants in nest gradually dismantled the prey items into small pieces. Secondly, wokers carried each fragment onto larva food basket which was the special organ for holding food. Then, larvae externally digested the fragment with saliva and enzymes. Finally, the workers continually checked whether the food was fully dissolved. After the digestion is completed, the wokers distributed digested prey tissue to colony members such as other wokers, queens and larvae by trophallaxis. The larvae that digested food did not ingest food until they were fed by workers (Cassill et al., 2005).

In this study, the baits were adjacently placed around the nest entrance and it was not to be a visual cue that made the ants to know where the baits existed. For example, in our daily life why do the ants usually know that where is the food we keep, despite it is kept very far from ants' nest compared with their body sizes. The answer is about smell that acts as a cue for ants. In insects, chemoreceptor responds for smelling called olfactory sensillum which densely located around antennae, maxillary and labial palps (Chapman, 2013; Klowden, 2013). Olfactory chemoreceptors respond to odorous molecules even if in very low concentrations or the smells orginate from far distance (Klowden, 2013). Most odor molecules are lipophilic, so they can dissolve dendritic membrane and these molecules are transported by general odor-binding proteins (OBPs) which are present in both sexes and less specific than pheromone bind-proteins (Chapman, 2013).

In current study, the results revealed that pork liver (piece) and ground pork liver (paste) were usually early detected by Singapore ants. This could be explained by the strong smell (in human perspective) of pork liver and ground pork liver. From the six bait choices, in human perception and prospect, we usually conceive that pork liver and ground pork liver pose more raw smell stronger than other bait choices as a result of the more competitive affinity (due to the more number of odor molecules from stronger smell) to bind with OBPs than other mild smells such as fat and sugar. However, the study of odor molecule density from various sources and olfactory chemoreceptors in insects still need further investigation which may be applied to use in bait formulation to efficiently attract ant pests.

5.4.2 Ant abundance

There were fluctuations of Singapore ant abundance among four ant colonies in one year. These may be the result of inevitable and intractable problems from human activities such as the alteration of microhabitat (e.g. gardening, landscape alteration and littering) and interspecific competition affecting ant abundance in some periods of the year. However, during the dry season, the number of foraging ants was slightly low which may be a consequence of unsuitable physical factors. For example, the low relative humidity in dry season may increase the risk of desiccation.

5.5 Conclusion

Overall, Singapore ants preferred carbohydrate and protein baits with different preferences in different seasons. During dry season, the most preferred bait was protein, while during wet season carbohydrate was the most preferred. Bait formulation also influenced bait preference of Singapore ants. The ants preferred solid baits over liquefied bait. The first detected bait by Singapore ants was not occurred by chance and likely to be occurred as a consequence of bait odors. It is likely that pork liver as well as ground pork liver have a good capacity of attracttion. In addition, Singapore ant abundance was slightly low during dry period of the year.

CHAPTER VI

Conclusion and recommendation

6.1 Conclusion

The foraging activity of Singapore ant generally peaked at 4 pm until 6 pm. There was no effect of sunrise and sunset times on the emergent or cessation of foraging times. Singapore ant foraging activity during daytime was significantly higher than during nighttime, and the ant foraging activity with bait also was greater than regular foraging activity.

Abiotic factors (physical factors) such as surface temperature and air temperature played an important role in Singapore ant activities. More Singapore ant number foraged in wet season than dry season which was a consequence of the difference in physical factors between seasons. The result from quasi-Poisson regression confirmed that surface temperature and air temperature influenced ant activity, while relative humidity did not show the clear effects. Results from species response curves clearly showed that Singapore ants foraged in the wider physical ranges when bait was present. Season also affected Singapore ant foraging range which was greater in dry season than wet season.

The communication for food location of Singapore ants principally relied on smelling sense that works with food odor and trail pheromone. Antenna contact and body contact were also crucial to amplify communication signal among worker ants. Closing nest entrance of Singapore ants only occurred if the nest entrance was hole located both on natural and man-made ground. Singapore ants can outcompete most ant species except the ghost ant, *T. melanocephalum*, which used chemical scent to subdue Singapore ants. Besides competition, Singapore ant also inhabited with silverfish which stole the ants' food in ant colony.

Basically, Singapore ants preferred both carbohydrate- and protein-based baits. The most preferred bait of Singapore ants in the dry season was protein, while the most preferred bait in the wet season was carbohydrate. The result strongly indicated that Singapore ant preferred solid bait over liquefied bait, and the ant might primarily sense based on the strong of bait odor. The monitoring of Singapore ant abundance under field condition showed the fluctuation in ant abundance throughout the year, but the ant abundance was slightly low during dry season.

6.2 Recommendations

1. Further study should be conducted on ant abundance which can be monitored "what happen inside the nest" (the number of queens, the number of larvae in different seasons, the stored food etc.) which could provide more robust evidences to use in discussion in foraging activity, food preference and ant abundance topics.
2. Solid bait formulation, mixture between carbohydrate and protein, should be applied to use in pest control due to the high preference of Singapore ant.
3. The most suitable time of the day for placing the bait is before the ant peak activity (4 pm-6pm) and the most appropriate time of the year for using bait is during dry season. These because bait can be applied in less quantity (lower cost) with the maximum efficiency for controlling ant's abundance.
4. Ant culture under laboratory condition may provide more implement results to interpret and compare with the results from field condition influenced by uncontrollable factors.
5. Dry season is the suitable time of the year that pest control should be conducted because of the low ant abundance.

REFERENCES

Abbott, K.L., Green, P.T. and O'Dowd, D.J. 2014. Seasonal shifts in macronutrient preferences in supercolonies of the invasive Yellow Crazy Ant *Anoplolepis gracilipes* (Smith, 1857) (Hymenoptera: Formicidae) on Christmas Island, Indian Ocean. Austral Entomology 53: 337-346.

Abril, S., Oliveras, J. and Gomez, C. 2007. Foraging activity and dietary spectrum of the Argentine ant (Hymenoptera: Formicidae) in invaded natural areas of the northeast Iberian Peninsula. Environmental Entomology 36: 1166–1173.

Agosti, D., Majer, J.D., Alonso, L.E. and Schultz, T.R. 2000. Ants: Standard Methods for Measuring and Monitoring Biodiversity. Washington D.C.: Smithsonian Institution Press.

Azcarate, F.M., Kovacs, E. and Peco, B. 2007. Microclimatic conditions regulate surface activity in harvester ants *Messor barbarus*. Journal of Insect Behavior 20: 315–329.

Bernstein, R.A. 1979. Schedules of foraging activity in species of ants. Journal of Animal Ecology 48: 921–930.

Bestelmeyer, B.T. 1997. Stress tolerance in some Chacoan dolichoderine ants: implications for community organization and distribution. Journal of Arid Environments 35: 297–310.

Bolton, B. 1987. A review of the *Solenopsis* genus-group and revision of Afrotropical *Monomorium* Mayr (Hymenoptera: Formicidae). Bulletin of the British Museum (Natural History) Entomology 54: 324–325.

Bolton, B. 1994. Identification Guide to the Ant Genera of the World. London: Harvard University Press.

Carroll, C.R. and Janzen, D.H. 1973. Ecology of foraging by ants. Annual Review of Ecology, Evolution, and Systematics 4: 231–257.

Cassill, D.L., Butler, J., Vinson, S.B. and Wheeler, D.E. 2005. Cooperation during prey digestion between workers and larvae in the ant, Pheidole spadonia. Insectes Sociaux 52: 339–343.

Cerda, X., Retana, J. and Cros, S. 1998. Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. Functional Ecology 12: 45–55.

Chapman, R.F. 2013. The Insects: Structure and Function. 5th. New York: Cambridge University Press.

Chong, K.F. and Lee, C.Y. 2006. Food preference and foraging activity of field population of a pest ant, Tapinoma indicum (Hymenoptera: Formicidae). Sociobiology 48: 875–883.

Chong, K.F. and Lee, C.Y. 2009. Infuences of temperature, relative humidity and light intensity on the foraging activity of field populations of the Long legged ant, *Anoplolepis gracilipes* (Hymenoptera: Formicidae). Sociobiology 54: 531–539.

Cogni, R. and Oliveira, P.S. 2004. Patterns in foraging and nesting ecology in the neotropical ant, *Gnamptogenys moelleri* (Formicidae, Ponerinae). Insectes Sociaux 51: 123–130.

Cole, B.J., Smith, A.A., Huber, Z.J. and Wiernasz, D.C. 2010. The structure of foraging activity in colonies of the harvester ant, *Pogonomyrmex occidentalis*. Behavioral Ecology 21: 337–342.

Cook, S.C., Eubanks, M.D., Gold, R.E. and Behmer, S.T. 2010. Colony-level macronutrient regulation in ants: mechanisms, hoarding and associated costs. Animal Behaviour 79: 429–437.

Cook, S.C., Eubanks, M.D., Gold, R.E. and Behmer, S.T. 2011. Seasonality directs contrasting food collection behavior and nutrient regulation strategies in ants. PLoS ONE 6: e25407.

Crosland, M.W.J. 1990. Variation in ant aggression and kin discrimination ability within and between colonies. Journal of Insect Behavior 3: 359–379.

Debout, G., Schatz, B., Elias, M. and Mckey, D. 2007. Polydomy in ants: what we know, what we think we know, and what remains to be done. Biological Journal of the Linnean Society 90: 319–348.

Dussutour, A. and Simpson, S.J. 2009. Communal nutrition in ants. Current biology 19: 740–744.

Eow, A.G.H. and Lee, C.Y. 2007. Comparaive nutritional preference of tropical pest ants, *Monomorium pharaonis*, *Monomorium floricola* and *Monomorium destructor* (Hymenoptera: Formicidae). Sociobiology 49: 165–186.

Gayahan, G.G. and Tschinkel, W.R. 2008. Fire Ants, Solenopsis invicta, Dry and Store Insect Pieces for Later Use. Journal of Insect Science 8: 1–9.

Glunn, F.J., Howard, D.F. and Tschinkel, W.R. 1981. Food preference in colonies of the Fire ant *Solenopsis invicta*. Insectes Sociaux 28: 217–222.

Gordon, D.M. 1995. The development of an ant colony's foraging range. Animal Behavior 49: 649–659.

Gordon, D.M. 2002. The regulation of foraging activity in red harvester ant colonies. The American Naturalist 159: 509–518.

Gordon, D.M., Dektar, K.N. and Wollman, N.P. 2013. Havester ant colony variation in foraging activity and response to humidity. PLoS ONE 8: e63363.

Gordon, D.M., Guetz, A., Greene, M.J. and Holmes, S. 2011. Colony variation in the collective regulation of foraging by harvester ants. Behavioral Ecology 22: 429–435.

Gordon, D.M., Holmes, S. and Nacu, S. 2008. The short-term regulation of foraging in harvester ants. Behavioral Ecology 19: 217–222.

Hahn, D.A. and Wheeler, D.E. 2002. Seasonal foraging activity and bait preferences of ants on Barro Colorado Island, Panama. Biotropica 34: 348–356.

Halantera, H., Strassmann, J.E., Carrillo, J. and Queller, D.C. 2009. Unicolonial ants: where do they come from, what are they and where are they going? Trends in Ecology and Evolution 24: 341–349.

Harkness, R.D. and Maroudas, N.G. 1985. Central place foraging by an ant (*Cataglyphis bicolor* Fab.): a model od searching. Animal Behaviour 33: 916–928.

Harris, R. (n.d.). Invasive Ant Risk Assessment. Wellington: Ministry for Primary Industries, New Zealand.

Harrison, J.S. and Gentry, J.B. 1981. Foraging pattern, colony distribution, and foraging range of the Florida harvester ant, *Pogonomyrmex badius*. Ecology 62: 1467–1473.

Hawkins, D. 2005. Biomeasurement: Understanding, Analysing, and Communicating Data in the Biosciences. New York: Oxford University Press.

Herrera, H.W. and Causton, C.E. 2010. First inventory of ants (Hymenoptera: Formicidae) on Baltra Island, Galapagos. Galapagos Research 67: 13–17.

Holland, S.M. 2014. Species response curves. [Online]. June 2014. Available from: http://strata.uga.edu/6370/handouts/SpeciesResponseCurves.pdf [28 July 2015]

Holland, S.M. and Zaffos, A. 2011. Niche conservatism along an onshore-offshore gradient. Paleobiology 37: 270–286.

Holldobler, B. and Wilson, E.O. 1990. The Ants. Massachusetts: Belknap Press.

Hood, W.G. and Tschinkel, W.R. 1990. Desiccation resistance in arboreal and terrestrial ants. Physiological Entomology 15: 23–35.

Hooper, L.M., Appel, A.G. and Rust, M.K. 2002. Preference of food particle size among several urban ant species. Journal of Economic Entomology 95: 1222–1228.

Hu, M.C., Pavlicova, M. and Nunes, E.V. 2011. Zero-inflated and hurdle models of count data with extra zeros: examples from an HIV-risk reduction intervention trial. The American Journal of Drug and Alcohol Abuse 37: 367–375.

Jaitrong, W. and Nabhitabhata, J. 2005. A List of Known Ant Species of Thailand (Formicidae: Hymenoptera). The Thailand Natural History Museum Journal 1: 9–54.

Jandt, J.M., et al. 2014. Behavioural syndromes and social insects: personality at multiple levels. Biological Reviews 89: 48–67.

Jayatilaka, P., Narendra, A.S.F., Reid, S.F., Cooper, P. and Zeil, J. 2011. Different effects of temperature on foraging activity schedules in sympatric *Myrmecia* ants. Journal of Experimental Biology 214: 2730–2738.

Kaspari, M. and Weiser, M.D. 2000. Ant activity along moisture gradients in a Neotropical forest. Biotropica 32: 703–711.

Kay, C.A.R. and Whitford, W.G. 1978. Critical thermal limits of desert honey ants: possible ecological implications. Physiological Zoology 51: 206–213.

Klowden, M.J. 2013. Physiological System in Insects. 3rd. San Diego: Academic Press.

Lach, L., Parr, C.L. and Abbott, K.L. 2010. Ant Ecology. Oxford: Oxford University Press.

Lee, C.Y. 2002. Tropical household ants: pest status, species diversity, foraging behavior, and baiting studies. Proceeding of the Fourth International Conference on Urban Pests, 3–18.

Lee, C.Y. 2008. Sucrose bait base preference of selected urban pest ants (Hymenoptera: Formicidae). The Sixth International Conference on Urban Pests, Hungary. 59–63.

Loke, P.Y. and Lee, C.Y. 2005. Effects of colony compositions and food type on foraging behavior of *Monomorium orientale* (Hymenoptera: Formicidae). Sociobiology 46: 595–602.

Mallis, A. 1997. Handbook of Pest Control. 8th ed. (n.p.): Mallis Handbook & Technical Training.

Markin, G.P. 1970. Food distribution within laboratory colonies of the Agentine ant, *Iridomyrmex humilis* (Mayr). Insect Sociaux 17: 127–158.

Mashaly, A.A.M., Mekhlafi, F.A.A. and Qahtini, A.M.A. 2013. Foraging activity and food preferences of the samsum ant, Pachycondyla sennaarensis. Bulletin of Insectology 66: 187–193.

Medeiros, F.N.S. and Oliveira, P.S. 2009. Season-Dependent Foraging Patterns Case Study of a Neotropical Forest-Dwelling Ant (*Pachycondyla striata*; Ponerinae). In S. Jarau and M. Hrncir (ed.), Food Exploitation By Social Insects: Ecological, Behavioral, and Theoretical Approaches, pp. 81-95. Boca Raton: Taylor & Francis Group.

Metcalf, R.L. and Luckmann, W.H. 1994. Introduction to Insect Pest Management. 3rd ed. New York: Wiley-Interscience.

Michael Glancey, B., Vander Meer, R.K., Glover, A., Lofgren, C.S. and Vinson, S.B. 1981. Filtration of microparticles from liquids ingested by the red imported fire antSolenopsis invicta Buren. Insectes Sociaux 28: 395–401.

Narendra, A., Reid, S.F. and Hemmi, J.M. 2010. The twilight zone: ambient light levels trigger activity in primitive ants. Proceedings of the Royal Society B 277: 1531–1538.

Nonacs, P. and Dill, L.M. 1988. Foraging response of the ant *Lasius pallitarsis to* food sources with associated mortality risk. Insectes Sociaux 35: 293–303

Norasmah, B., Abu Hassan, A., Che Salmah, M.R., Nurita, A.T. and Nur Aida, H. 2006. Daily foraging pattern and proteinaceous food preferences of Solenopsis geminata (Fabricius) (Hymenoptera: Formicidae). Tropical Biomedicine 23: 134–139.

Orr, M.R. and Seike, S.H. 1998. Parasitoids deter foraging by Argentine ants (Linepithema humile) in their native habitat in Brazil. Oecologia 117: 420–425.

Petralia, R.S. and Vinson, S.B. 1979. Comparative anatomy of the ventral region of ant larvae, and its relation to feeding behavior. Psyche 86: 375–394.

Pinter-Wollman, N. 2012. Personality in social insects: How does worker personality determine colony personality? Current Zoology 58: 580–588.

Pinter-Wollman, N., Gordon, D.M. and Holmes, S. 2012. Nest site and weather affect the personality of harvester ant colonies. Behavioral Ecology 23: 1022–1029.

Porter, S.D. and Tschinkel, W.R. 1993. Fire ant thermal preferences: behavioral control of growth and metabolism. Behavioral Ecology and Sociobiology 32: 321–329.

Raimundo, R.L.G., Freitas, A.V.L. and Oliveira, P.S. 2009. Seasonal Patterns in Activity Rhythm and Foraging Ecology in the Neotropical Forest-Dwelling Ant, Odontomachus chelifer (Formicidae: Ponerinae). Annals of the Entomological Society of America 102: 1151–1157.

Rodr´ıguez, G. 2013. Models for Count Data With Overdispersion. [Online]. 6 November 2013. Available from: http://data.princeton.edu/wws509/notes/c4a.pdf [28 July 2015]

Schmid-Hempel, P. 1984. Individually different foraging methods in the desert ant *Cataglyphis bicolor* (Hymenoptera, Formicidae). Behavioral Ecology and Sociobiology 14: 263–271.

Schneirla, T.C. 1938. A theory of army-ant behavior based upon the analysis of activities in a representative species. Journal of Comparative Psychology 25: 51–90.

Sengupta, P., Ghorai, N. and Mukhopadhyay, S. 2010. Food preference and foraging of Fire ant *Solenopsis nitens*. Proceedings of the Zoological Society 63: 73–77.

Shattuck, S.O. 1992. Review of the Dolichoderine ant genus *Iridomyrmex* Mayr with descriptions of three new genera (Hymenoptera: Formicidae). Australian Journal of Entomology 31: 13–18.

Sinervo, B. 2013. Optimal Foraging Theory: Constraints and Cognitive Processes. [Online]. Availablefrom:http://bio.research.ucsc.edu/~barrylab/classes/CHAPTER_PDFS/Chap_6_Optimal.pdf [6 February 2016]

Smith, M.R. 1965. House-infesting Ants of the Eastern United States: Their Recognition, Biology, and Economic Importance. Washington: U.S. department of agriculture technical bulletin.

Stein, M.B., Thorvilson, H.G. and Johnson, J.W. 1990. Seasonal changes in bait preference by red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). The Florida Entomologist 73: 117–123.

Talbot, M. 1943. Response of the ant *Prenolepis imparis* Say to temperature and humidity changes. Ecology 24: 345–352.

Tomalski, M.D., Blum, M.S., Jones, T.H., Fales, H.M., Howard, D.F. and Passera, L. 1987. Chemistry and functions of exocrine secretions of the ants *Tapinoma melanocephalum* and *T. erraticum.* Journal of Chemical Ecology 13: 253–263.

Tsutsui, N.D. and Suarez, A.V. 2003. The Colony Structure and Population Biology of Invasive Ants*.* Conservation Biology 17: 48–58.

von Beeren, C., Schulz, S., Hashim, R. and Witte, V. 2011. Acquisition of chemical recognition cues facilitates integration into ant societies*.* BMC Ecology 11: 30.

Vowles, D.M. 1995. The foraging of ants*.* The British Journal of Animal Behaviour 3: 1–13.

Walter, H., Harnickell, E. and Mueller-Dombois, D. 1975. Climate-diagram Maps of the Individual Continents and the Ecological Climatic Regions of the Earth: Supplement to the Vegetation Monographs. Berlin: Springer Verlag.

Walters, A.C. and Mackay, D.A. 2003. An experimental study of the relative humidity preference and survival of the Argentine ant, *Linepithema humile* (Hymenoptera, Formicidae): comparisons with a native *Iridomyrmex* species in South Australia*.* Insectes Sociaux 50: 355–360.

Ward, P.S., Brady, S.G., Fisher, B.L. and Schultz, T.R. 2015. The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae)*.* Systematic Entomology 40: 61–81.

Wehne, R. and Muller, M. 2006. The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation*.* PNAS 103: 12575–12579.

Wetterer, J.K. 2009. Worldwide spread of the destroyer ant, *Monomorium destructor* (Hymenoptera: Formicidae)*.* Myrmecological News 12: 97–108.

Wetterer, J.K. and Espadaler, X. 2010. Invasive ants of Macaronesia. In Serrano, et al. (ed.), Terrestrial Arthropods of Macaronesia - Biodiversity, Ecology and Evolution, pp. 139. Lisbon: Sociedade de Industria Grafica.

Wiernasz, D.C. and Cole, B.J. 2010. Patriline shifting leads to apparent genetic caste determination in harvester ants*.* PNAS 107: 12958–12962.

Wright, T.F., Eberhard, J.R., Hobson, E.A., Avery, M.L. and Russello, M.A. 2010. Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. Ethology Ecology & Evolution 22: 393–404.

Zeileis, A., Kleiber, C. and Jackman, S. 2008. Regression models for count data in R. Journal of Statistical Software 27: 1–25.

APPENDIX

Appendix A

Table A-1 The significant differences of the ant numbers caught by five baiting in each paired bait type in preliminary study. (Man-Whitney U test, $p < 0.05$)

Paired baits	*P-value*
Peanut-Cookie	0.564
Peanut-Honey	0.189
Peanut-Jam	0.091
Peanut-Tuna	0.384
Cookie-Honey	0.020**
Cookie-Jam	0.018**
Cookie-Tuna	0.081
Honey-Jam	0.439
Honey-Tuna	0.559
Jam-Tuna	0.074

** Significant difference $p < 0.05$

Appendix B

Table B-1 The significant differences of the ant numbers between paired hours in activity without bait and activity with bait in one year round. (Man-Whitney U test, $p < 0.05$)

Paired hour in activity without bait and activity with bait	*P-value*
8:00	< 0.001**
10:00	< 0.001**
12:00 (noon)	< 0.001**
14:00	< 0.001**
16:00	< 0.001**
18:00	< 0.001**
20:00	< 0.001**
22:00	< 0.001**
0:00 (midnight)	< 0.001**
2:00	< 0.001**
4:00	< 0.001**
6:00	= 0.003**

** Significant difference $p < 0.05$

Table B-2 The significant differences of the ant numbers between paired hours in activity without bait and activity with bait in dry season. (Man-Whitney U test, $p < 0.05$)

Paired hour in activity without bait and activity with bait	*P-value*
8:00	= 0.010**
10:00	< 0.001**
12:00 (noon)	< 0.001**
14:00	< 0.001**
16:00	= 0.001**
18:00	= 0.078
20:00	= 0.103
22:00	= 0.160
0:00 (midnight)	= 0.204
2:00	= 0.343
4:00	= 0.075
6:00	= 0.176

** Significant difference $p < 0.05$

Table B-3 The significant differences of the ant numbers between paired hours in activity without bait and activity with bait in wet season. (Man-Whitney U test, $p < 0.05$)

Paired hour in activity without bait and activity with bait	*P-value*
8:00	< 0.001**
10:00	< 0.001**
12:00 (noon)	< 0.001**
14:00	< 0.001**
16:00	< 0.001**
18:00	< 0.001**
20:00	< 0.001**
22:00	< 0.001**
0:00 (midnight)	< 0.001**
2:00	< 0.001**
4:00	= 0.001**
6:00	= 0.007**

** Significant difference $p < 0.05$

Appendix C

Table C-1 The significant differences of the ant numbers caught by six baiting in each paired bait type in one year round in food preference study. (Man-Whitney U test, $p < 0.05$)

Paired baits	*P-value*
Sucrose syrup - Sucrose agar	< 0.001**
Sucrose syrup - Pork liver	0.001
Sucrose syrup - Ground pork liver	0.187
Sucrose syrup - Pork fat	0.896
Sucrose syrup - Lard	< 0.001**
Sucrose agar - Pork liver	0.289
Sucrose agar - Ground pork liver	< 0.001**
Sucrose agar - Pork fat	< 0.001**
Sucrose agar - Lard	< 0.001**
Pork liver- Ground pork liver	< 0.001**
Pork liver- Pork fat	0.002
Pork liver- Lard	< 0.001**
Ground pork liver- Pork fat	0.189
Ground pork liver- Lard	< 0.001**
Pork fat- Lard	< 0.001**

** Significant difference $p < 0.05$

Table C-2 The significant differences of the ant numbers caught by six baiting in each paired bait type in dry season in food preference study. (Man-Whitney U test, $p < 0.05$)

Paired baits	*P-value*
Sucrose syrup - Sucrose agar	0.217
Sucrose syrup - Pork liver	0.002**
Sucrose syrup - Ground pork liver	0.152
Sucrose syrup - Pork fat	0.058
Sucrose syrup - Lard	< 0.001**
Sucrose agar - Pork liver	0.047**
Sucrose agar - Ground pork liver	0.746
Sucrose agar - Pork fat	0.659
Sucrose agar - Lard	< 0.001**
Pork liver- Ground pork liver	0.015**
Pork liver- Pork fat	0.104
Pork liver- Lard	< 0.001**
Ground pork liver- Pork fat	0.340
Ground pork liver- Lard	< 0.001**
Pork fat- Lard	< 0.001**

** Significant difference $p < 0.05$

Table C-3 The significant differences of the ant numbers caught by six baiting in each paired bait type in wet season in food preference study. (Man-Whitney U test, $p < 0.05$)

Paired baits	*P-value*
Sucrose syrup - Sucrose agar	< 0.001**
Sucrose syrup - Pork liver	0.092
Sucrose syrup - Ground pork liver	0.018**
Sucrose syrup - Pork fat	0.225
Sucrose syrup - Lard	< 0.001**
Sucrose agar - Pork liver	0.005**
Sucrose agar - Ground pork liver	< 0.001**
Sucrose agar - Pork fat	< 0.001**
Sucrose agar - Lard	< 0.001**
Pork liver- Ground pork liver	< 0.001**
Pork liver- Pork fat	0.007**
Pork liver- Lard	< 0.001**
Ground pork liver- Pork fat	0.291
Ground pork liver- Lard	< 0.001**
Pork fat- Lard	< 0.001**

** Significant difference $p < 0.05$

VITA

Mr. Ussawit Srisakrapikoop was born on August 28, 1990. After completing his study at Phrapathom Witthayalai School in 2008, he entered Silpakorn University for a Bachelor's degree from the Department of Biology, Faculty of Science and graduated from there with first-class honors. In 2013, he continued his Master degree of Science, Zoology program in Department of Biology, Faculty of Science, Chulalongkorn University and completed the program in 2016. His research was supported by Development and Promotion of Science and Technology Talents Project (DPST).

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