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Mortality and Repellent Effects of Coffee Extracts on The Workers of Three Household Ant Species

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ABSTRACT

Coffee consists of a variety of chemical compounds that has not been documented to have resistance on insects. Hence, this research was conducted to study the impact of coffee extracts impregnated in gel bait towards survival and feeding behaviour of *Tapinoma indicum* (ghost ant), *Pheidole megacephala* (big-headed ant) and *Monomorium pharaonis* (Pharaoh ant) (Hymenoptera: Formicidae). The three coffee species used were *Coffea arabica*, *Coffea canephora* and *Coffea liberica*. The coffee extracts were obtained using Soxhlet extraction method, diluted to 0.01%, 0.05% and 0.10% concentration, and eventually impregnated into two sets of gel bait at with the first set (Set I) sugar solution and the second set (Set II) with distilled water. The overall results indicated that *Coffea arabica* gave highest mortality on all three ant species and higher concentration of extracts showed higher ant mortality in most bioassays. The higher mortality in lower concentration bioassays was probably due to their lower repellency percentages. Furthermore, Set I bioassays had higher mortality as the sugar used act as food attractant. *T. indicum* was the most susceptible species. Owing to the low mortality, the low concentration of coffee used was not effective in killing household ants but it did repel them.

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INTRODUCTION

Ants are known to be an ecologically dominant group and show the highest level of diversity among eusocial insects (Wilson & Hölldobler, 2005). They involve in the interactions with other organisms and also the functional ecosystem processes (Wilson & Hölldobler, 2005). With the exception of Antarctica and Arctic, their dominance is indicated by their worldwide geographical distribution. Each ant species possesses its own particular morphological structures and behaviours, making it distinguishable from other ant species (Lucky, 2009).

Furthermore, ants are one of the most nuisance urban pests when they enter from outdoors to indoors in searching of food and water. Due to their properties of large number appearance, cause contamination of food and hospital sterile equipments, they are considered as nuisance pests and disease organism carriers which make them to be recognized as potential mechanical vectors of human diseases (Beatson, 1972). Some ant species have the ability to cause painful bites or stings with their pincer-like jaws or venomous stings (Marer & Flint, 1991). Household ants can be discovered with a higher frequency in tropical areas compared to temperate areas (Campos-Farinha, 2005; Fowler, Filho, & Bueno, 1993).

In Asia, the pest status of household ants was less significant in the 1990s. However, this situation had soon changed owing to the rise of its pest status (Lee, 2000). In the pest control company of Malaysia, around 10% of the business was constituted by the controlling of ant in 1995 (Na & Lee, 2001). While in United States, the ant control revenue of ant is so high that the ants have attained the top household pest status (Gooch, 1999; Jenkins, 2001; Kaminski, 2000) and ranked the most troublesome pest (Gooch, 1999). From a residential survey, which was carried out in 1995, ants have attained the status of the most important household pests after mosquitoes and cockroaches (Na & Lee, 2001). In Malaysia, there are 23 species of household ants with a total of 15 genera described (Na & Lee, 2001). However, in this research, only three common species of household ants are focused: *Tapinoma indicum* (Forel) (ghost ant), *Pheidole megacephala* (F.) (big-headed ant) and *Monomorium pharaonis* (L.) (Pharaoh ant) (Hymenoptera: Formicidae).

There are several methods to control the household ants. Baiting and residual spraying are the common methods for controlling ants (Lee, 2000), but baiting has served as a more popular method due to its usage safety, target-specific and ability to eliminate or suppress the whole ant colony without the requirement to locate the nest (Suiter, Wu, & Bennett, 1997). Generally, baits are more effective against household ants as many residual contact insecticides used act repellent to ants, especially Pharaoh's ants (Gooch, 1999). Residual insecticide treatment just acts as barriers of preventing ants from entering the houses instead of eliminating the ant population (Klotz, Greenberg, Shorey, & Williams, 1997). Hence, this method is not effective against some species of household ants which reside within the house (Lee, 2000).

Recent studies have shown that plants such as coffee and tea are used as effective biological agent in controlling insects (Ab Majid et al., 2018). Coffee consists of over 1000 chemical compounds (Farah, 2012); while few *Coffea* species are resistant to insect attack naturally (Jaramillo, Borgemeister, & Baker, 2006). Coffee has been utilized to study toxicological effects on several organisms. Caffeine causes damage to the nervous system in bullfrog (Higure & Nohmi, 2002), blocks the fetal development of *Rattus norvegicus* (Smith, McElhatton, & Sullivan, 1987) and inhibits oviposition of shot-hole borer beetle (Hewavitharanage, Karunaratne, & Kumar, 1999). Coffee is known to be a natural repellent to ants at which ants repel when contact with the coffee grounds. Few researches have been reported that coffee is effective in decreasing the mosquitoes' reproductive capacity (Laranja, Manzatto, & de Campos Bicudo, 2003), repelling gravid *Aedes albopictus* female and inhibiting the development of their embryos (Satho et al., 2015).

In this study, three species of coffee: *Coffea arabica* (Arabica coffee), *Coffea canephora* (Robusta coffee) and *Coffea liberica* (Liberian coffee) are extracted by using Soxhlet extraction and impregnated in the gel bait to test their impact in controlling household ants. The coffee species used are roasted type in which they are not mixed with sugar to avoid creating bias towards the attraction of ants. The objectives of this study are, to investigate the effect of the extracts of *C. arabica*, *C. canephora* and *C. liberica* impregnated in gel bait towards survival of the workers of *T. indicum*, *P. megacephala* and *M. pharaonis*.

MATERIALS AND METHODS

Coffee Source

The coffee beans of *C. arabica*, *C. canephora* and *C. liberica* were obtained from Cap Kuda Coffee Company, Sabah, Malaysia. The coffee beans were roasted without adding any sugar compounds. The temperatures used in the roasting process vary from 210°C to 240°C and the roasting time used was about 12 to 30 min. These roasted coffee beans were then ground, packaged and shipped to Universiti Sains Malaysia.

Extract Coffee Using Soxhlet Extraction

A small cotton ball was moistened with water and placed in the chamber of Soxhlet extractor. Fifty grams of each of the roasted coffee (*C. arabica, C. canephora* and *C. liberica)* were weighted and placed separately into the Soxhlet extractor. A volume of 250 ml of methanol that used as the extraction solvent was poured into the flat-bottomed flask. The flat-bottomed flask was then placed on the heating mantle; the Soxhlet extractor together with the reflux condenser was placed atop of the flat-bottomed flask. The Soxhlet extractor was fixed and held by retort stand. Both ends of the reflux condenser were connected to pipes for water in and water out.

When the apparatus was ready, the extraction solvent (methanol) was heated

until its boiling point (64.7 °C) was achieved. Its vapour condensed in the condenser and the condensed extractant dripped into the chamber containing the coffee. When the liquid level in the chamber had risen to the top of the siphon tube, the extract-containing solvent of the Soxhlet chamber were siphoned into the flat-bottomed flask. The whole apparatus was heated for 5 h (Mgbemena, Ebe, Nnadozie, & Ekeanyanwu, 2015).

After 5 h, the entire apparatus was left to be cooled down. The coffee extracts were then collected and poured to a glass petri dish with correct label. The extracts were then placed into drying oven (Memmert GmbH + Co, KG, Western Germany) at 80 °C for evaporation for three days to obtain the coffee extract in solute form. The coffee extracts were then taken out and scraped off by using spatula. The scraped coffee extracts were kept in universal bottle with labeling and then stored in refrigerator for further used.

Collection and Identification of the *T. indicum, P. megacephala* and *M. pharaonis*

Field populations of *T. indicum*, *P. megacephala* and *M. pharaonis* workers were collected from the Minden campus of Univeristi Sains Malaysia, Penang, Malaysia from 7:00 a.m. to 10:00 a.m. The traps were set up by using Eppendorf tubes (with modified holes on the tubes) at which the inner surface of the Eppendorf tubes was coated with a thin layer of Fluon, polytetrafluroethylene suspension (BioQuip Products, Inc., California) to prevent the trapped ants from escaping (Eow, Chong, & Lee, 2004). A minute amount of peanut butter or honey which acts as food attractant to ants was placed on a small piece of paper and inserted it into the Eppendorf tubes. The trapped ants were collected after 1 to 2 h and transferred into container at which the inner surface was coated with fluon. The ants were put in 90% ethanol for identification according to their distinct characteristics based on descriptions by Na and Lee (2001) and Lucky (2009). A brush was used to separate the ant species if more than one ant species were trapped in the same tube.

Preparation of Gel Bait by Using Different Concentration of Coffee Extracts

The coffee extract solution with desired concentration was prepared by mixing coffee extracts and distilled water or 20% of sugar solution. Preparation of coffee concentration. i.e. 0.01%, 0.05% and 0.10% were produced using Arabica, following method of Ab Majid et al. (2018) with slight modification. To allow the coffee extracts to dissolve completely in the solution, the solution was allowed to stir by using magnetic stirrer for 30 to 45 min. After all, the gelling agent, Ferti-plant jelly (Fertiland Trading Co., Malaysia) was added into the prepared solution, allowing it to absorb the solution and expand to its maximum size for 12 h.

Two sets of gel baits were prepared. The first set of gel was the mixture of coffee extract and 20% of sugar solution (Set I).

The scraped coffee extract was diluted to different concentration such that 0.01% (low), 0.05% (medium) and 0.10% (high) by using 100 ml of 20% of sugar solution. The blank bait (control) used for this set contained only 20% of sugar solution. The second set of gel was the mixture of coffee extract and distilled water (Set II). The scraped coffee extract was diluted to different concentration such that 0.01% (low), 0.05% (medium) and 0.10% (high) by using 100 ml of distilled water. The blank bait (control) used consists of only distilled water. All of the gels were made constant mass of 0.50 g by using weighing machine.

Bioassay

A small hole (5 mm in diameter) was made at the center of the petri dish lid by using a hot soldering iron (Williams, 1989). This was to insert the cotton wool moistened with distilled water (without touching the base of petri dish) as moisture for ants (Figure 1)

A 90 mm diameter filter paper was attached to the outer base of petri dish to ease the counting process. The perimeter of the petri dish inner surface was coated with a thin layer of petroleum jelly (Vasline, Unilever Thai, Thailand) (Figure 1). Thirty ant workers were randomly picked and transferred to a petri dish (90 mm in diameter). The first set of gel bait was placed in the petri dish (Set I). Gel bait with only 20% of sugar solutions (without any coffee extracts) was used as the control of the experiment (Figure 2). Parafilm was used to seal up the petri dish to prevent the ants from escaping.

The ants were observed in 30 min, 1 h, 2 h, 4 h, 8 h, 24 h (1 day), 48 h (2 days) and 72 h (3 days) under temperature of 25 ± 2°C and relative humidity of 76 ± 10%. The number of ants which cannot move or respond (ant mortality) were counted and recorded. Their repellency and behaviours were also observed and recorded under dissecting microscope. The number of ants that were not attracted to the region with gel bait during the observed time was considered as repelling.

The above steps were repeated to complete three sets of replicates for each ant species with different concentrations (0.01%, 0.05% and 0.10%) for each coffee species. The bioassay for the second set of gel bait was conducted using the same procedures.

The mean repellency percentage for 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h and 72 h; and mean mortality were obtained. The significant differences of mortality and repellency were determined using Kruskal-Wallis (KW) analysis of variance by SPSS 22.0 software. The repellency percentage (PR) was calculated using the formula (Abdullah et al., 2015):

$$PR = \frac{NC - NT}{NC + NT} \times 100$$

where,

NC = Number of ants on the region without gel bait

NT = Number of ants on the region with gel bait

RESULTS

Effect of Coffee Extracts Impregnated in Gel Bait Towards Survival of *T. indicum, P. megacephala* And *M. pharaonis*

C. canephora and *C. liberica* showed insignificant result (P>0.05) with P value 0.109 and 0.054 respectively against *T. indicum* between the three concentrations (0.01%, 0.0% and 0.10%) and control in Set I bioassay (Figure 1). *C. arabica* showed significant results (P<0.05) with P value 0.032 against *T. indicum* between the treatments and control in Set I bioassay but no significant differences among the three concentrations (Figure 1). In Set II bioassay, *C. canephora* displayed insignificant results (P>0.05) with P value 0.079 against *T. indicum*; while *C. arabica* and *C. liberica* had significant results (P<0.05) with P values 0.044 and 0.030 respectively against *T. indicum* between treatments and control but no significant differences among the three treatments (Figure 2).

In Set I bioassay, *P. megacephala* displayed the same results with those of *T. indicum* for the similar set. Both *C. canephora* and *C. liberica* showed insignificant result (P>0.05) with P values 0.144 and 0.114 respectively between the concentrations and control (Figure 3). *C. arabica* indicating significant results (P<0.05) with P value 0.030 between the treatments and control but no significant difference among the three concentrations (Figure 3). In Set II bioassay, there were no significant results (P>0.05) for all the three coffee species (*C. arabica* P=0.067; *C. canephora* P=0.127; and *C. liberica* P=0.392) against *P. megacephala*(Figure 4).

For *M. pharaonis*, all three coffee species (*C. arabica* P=0.134; *C. canephora* P=0.144; *C. liberica* P=0.212) had insignificant results (P>0.05) between the three concentrations and control in Set I bioassay (Figure 5). In Set II bioassay, there were insignificant results (P>0.05) for *C. arabica* (P=0.132) and *C. liberica* (P=0.441) between the concentrations and control. On the other hand, *C. canephora* (P=0.048) showed a significant difference (P<0.05). Nevertheless, it did not display significant results among the three concentrations (Figure 6).

At all concentrations in both sets, *C. arabica*, *C. canephora* and *C. liberica* showed insignificant results (P>0.05) among themselves against *T. indicum* with the exception of 0.05% concentration of Set II bioassay (Figures 1 and 2). Instead, its P value of 0.047 displayed a significant difference of the ant mortality among the coffee species at 0.05% at which *C. arabica* and *C. canephora* significantly differed from *C. liberica*. For *P. megacephala*, 0.01% showed insignificant results (P>0.05) among the three coffees with P value 0.141 and 0.100 in Set I and Set II bioassay respectively. However, 0.05% and 0.10% in Set I bioassay indicated significant results (P<0.05) among the coffees with P value 0.042 and 0.048 respectively, at which *C. arabica* differed significantly to both *C. canephora* and *C. liberia* (Figure

3). For Set II bioassay, the result (P=0.088, P>0.05) showed no significant difference at 0.05% among the three *Coffea* spp.; but it showed that *C. arabica* (P=0.034, P<0.05) experienced significant difference to *C. canephora* and *C. liberica* at 0.10% (Figure 4). The analysis for *M. pharaonis* showed that at all concentrations, there were no significant differences among the coffees in both sets of bioassays (Set I: P=0.633 at 0.01%, 0.612 at 0.05% and 0.966 at 0.10%; Set II: P=0.264 at 0.01%, 0.641 at 0.05% and 0.396 at 0.10%) (Figure 5).

There were no significant results (P>0.05) of mean repellency percentage at all observed time intervals (30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h and 72 h) in *T. indicum* between the treatments and control of *C. arabica* and *C. liberica* in both Set I and Set II bioassays.

C. canephora had significant differences at 24 h (P=0.037) and 72 h (P=0.037) in Set I bioassay; and at 48 h (P=0.031) in Set II bioassay between concentrations and control (Tables 1 and 2).

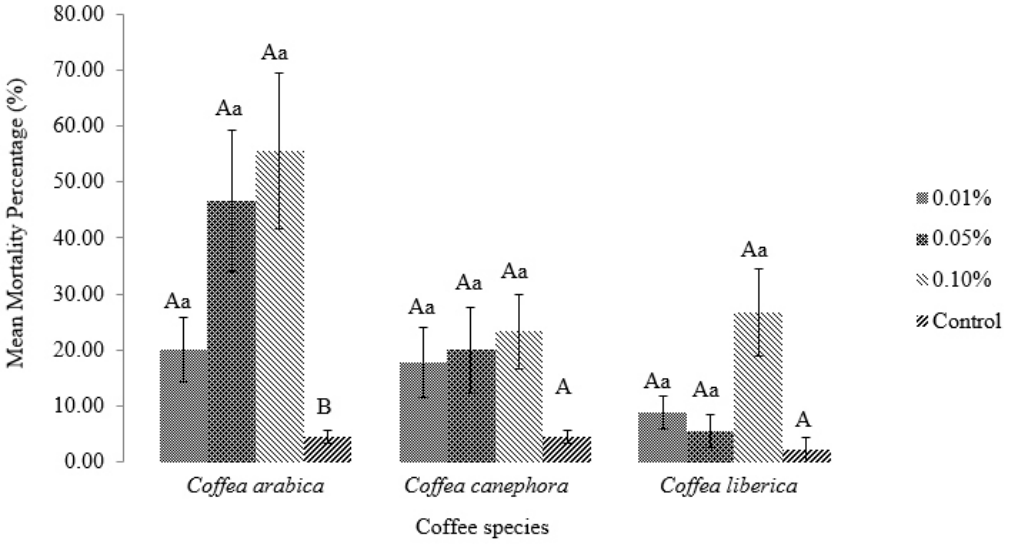

Figure 1. Mean mortality percentage of three different coffees against *T. indicum* in Set I bioassay

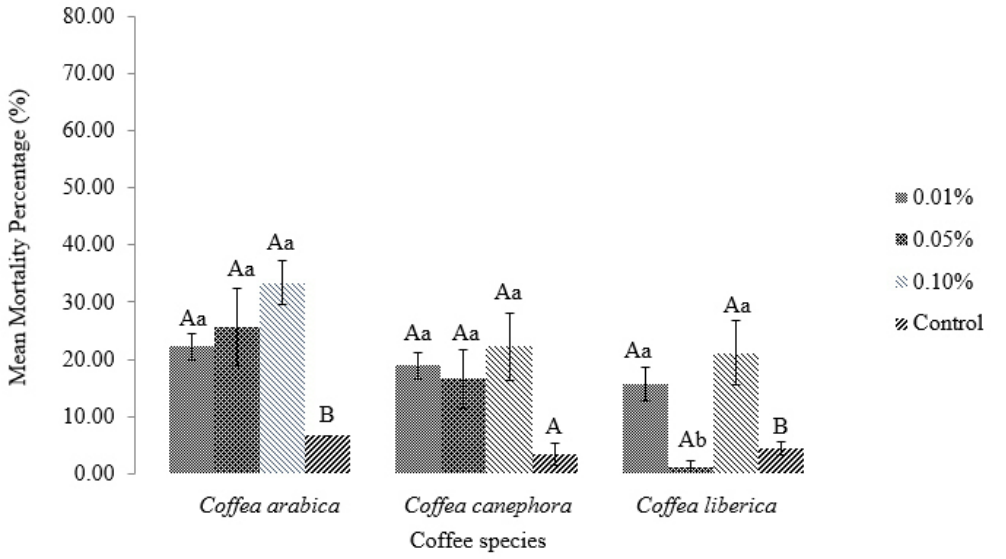

Figure 2. Mean mortality percentage of three different coffees against *T. indicum* in Set II bioassay

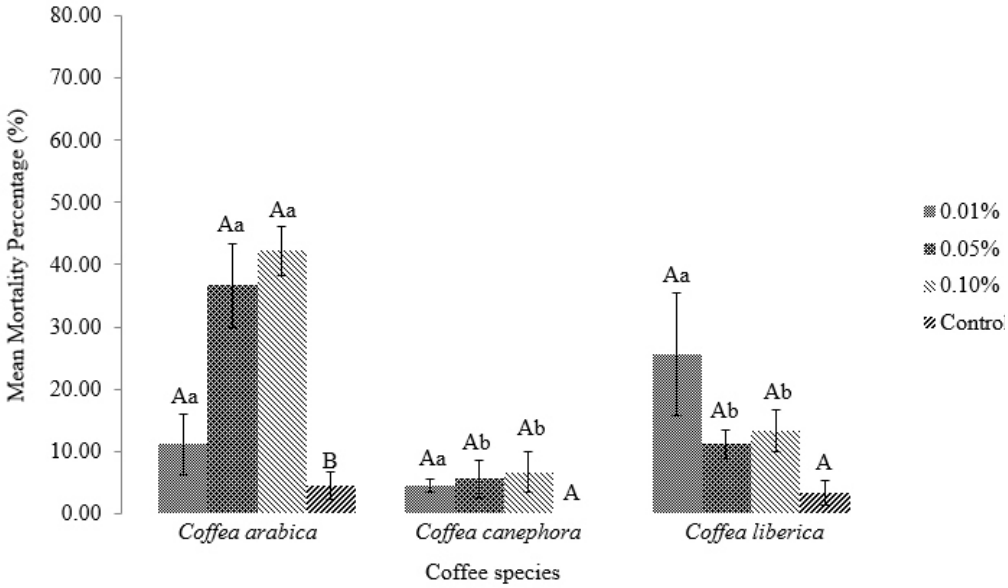

Figure 3. Mean mortality percentage of three different coffees against *P. megacephala* in Set I bioassay

Table 1

Mean mortality percentage and mean repellency percentage of three different coffees against T. indicum *in Set I bioassay*

Coffee species	Concentration (%)	Mean Mortality Percentage (%)[1]	Mean Repellency Percentage (%)[2]							
			30min	1h	2h	4h	8h	24h	48h	72h
Coffea arabica	**0.01%**	20.00 ± 5.77Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	95.55 ± 2.22C	100.00 ± 0.00C
	0.05%	46.67 ± 12.62Aa	100.00 ± 0.00C	95.56 ± 4.44C	93.33 ± 6.67C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	0.10%	55.55 ± 13.92Aa	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C
	Control	4.44 ± 1.11B	86.67 ± 10.18C	91.11 ± 8.89C	95.56 ± 4.44C	95.55 ± 2.22C	93.33 ± 3.85C	88.89 ± 5.88C	91.11 ± 5.88C	95.56 ± 4.44C
Coffea canephora	**0.01%**	17.78 ± 6.19Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C
	0.05%	20.00 ± 7.70Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C
	0.10%	23.33 ± 6.67Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C
	Control	4.44 ± 1.11A	95.55 ± 2.22C	93.33 ± 3.85C	97.78 ± 2.22C	93.33 ± 3.85C	95.55 ± 2.22C	88.89 ± 4.44D	86.67 ± 3.85C	88.89 ± 4.44D

Table 1 *(Continue)*

Coffea liberica	0.01%	8.89 ± 2.94Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	95.56 ± 4.44C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	0.05%	5.56 ± 2.94Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	91.11 ± 8.89C	97.78 ± 2.22C
	0.10%	26.67 ± 7.70Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	95.55 ± 2.22C	97.78 ± 2.22C
	Control	2.22 ± 2.22A	100.00 ± 0.00C	95.55 ± 2.22C	97.78 ± 2.22C	91.11 ± 4.44C	91.11 ± 5.88C	86.67 ± 6.67C	84.44 ± 4.44C	86.67 ± 7.70C

[1] Mean mortality percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: A & B (comparison of concentration within the same coffee species); a & b (comparison of coffee species within a concentration).

[2] Mean repellency percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: C, D & E (comparison of concentration within the same coffee species).

Table 2

Mean mortality percentage and mean repellency percentage of three different coffees against T. indicum *in Set II bioassay*

Coffee species	Concentration (%)	Mean Mortality Percentage (%)[1]	Mean Repellency Percentage (%)[2]							
			30min	1h	2h	4h	8h	24h	48h	72h
Coffea arabica	0.01%	22.22 ± 2.22Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C
	0.05%	25.56 ± 6.76Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	97.78 ± 2.22C	97.78 ± 2.22C	95.55 ± 4.45C	100.00 ± 0.00C
	0.10%	33.33 ± 3.85Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	97.78 ± 2.22C
	Control	6.67 ± 0.00B	97.78 ± 2.22C	95.56 ± 4.44C	100.00 ± 0.00C	95.55 ± 2.22C	95.56 ± 4.44C	91.11 ± 4.44C	95.56 ± 4.44C	84.44 ± 12.37C
Coffea canephora	0.01%	18.89 ± 2.22Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	95.55 ± 2.22C	97.78 ± 2.22C	97.78 ± 2.22C	100.00 ± 0.00C
	0.05%	16.67 ± 5.09Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	91.11 ± 8.89C
	0.10%	22.22 ± 5.88Aa	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	Control	3.33 ± 1.93A	97.78 ± 2.22C	95.55 ± 2.22C	97.78 ± 2.22C	95.56 ± 4.44C	93.33 ± 0.00C	93.33 ± 6.67C	88.89 ± 2.22D	88.89 ± 5.88C

Table 2 *(Continue)*

Coffea liberica	**0.01%**	15.56 ± 2.94Aa	100.00 ± 0.00	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C
	0.05%	1.11 ± 1.11Ab	100.00C ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C
	0.10%	21.11 ± 5.56Aa	97.78 ± 2.22C	97.78 ± 2.22C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	Control	4.44 ± 1.11B	97.78 ± 2.22C	95.56 ± 4.44C	100.00 ± 0.00C	97.78 ± 2.22C	95.56 ± 4.44C	97.78 ± 2.22C	95.56 ± 4.44C	93.33 ± 3.85C

[1] Mean mortality percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: A & B (comparison of concentration within the same coffee species); a & b (comparison of coffee species within a concentration).

[2] Mean repellency percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: C, D & E (comparison of concentration within the same coffee species).

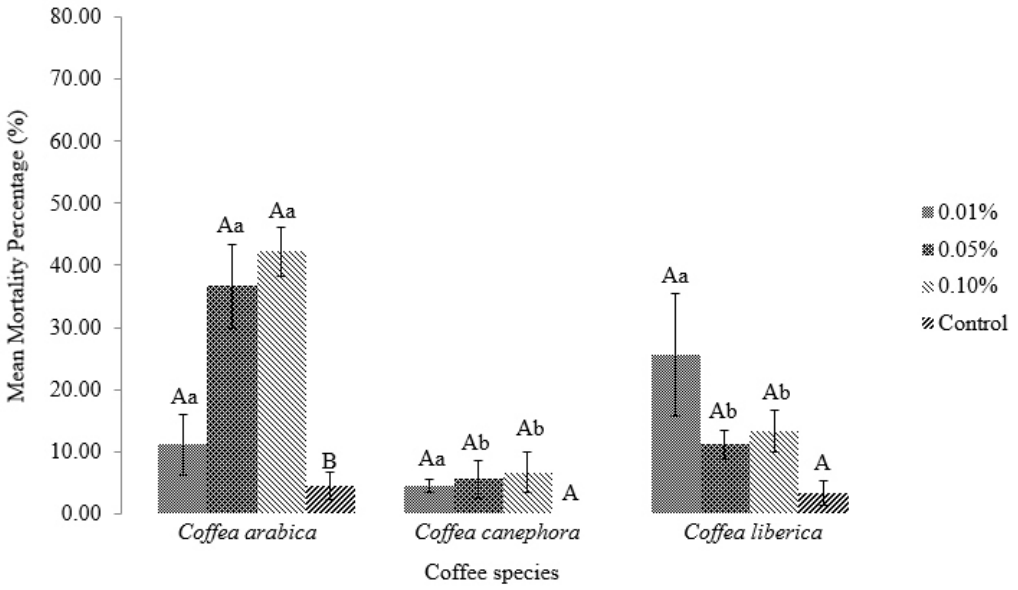

Figure 3. Mean mortality percentage of three different coffees against *P. megacephala* in Set I bioassay

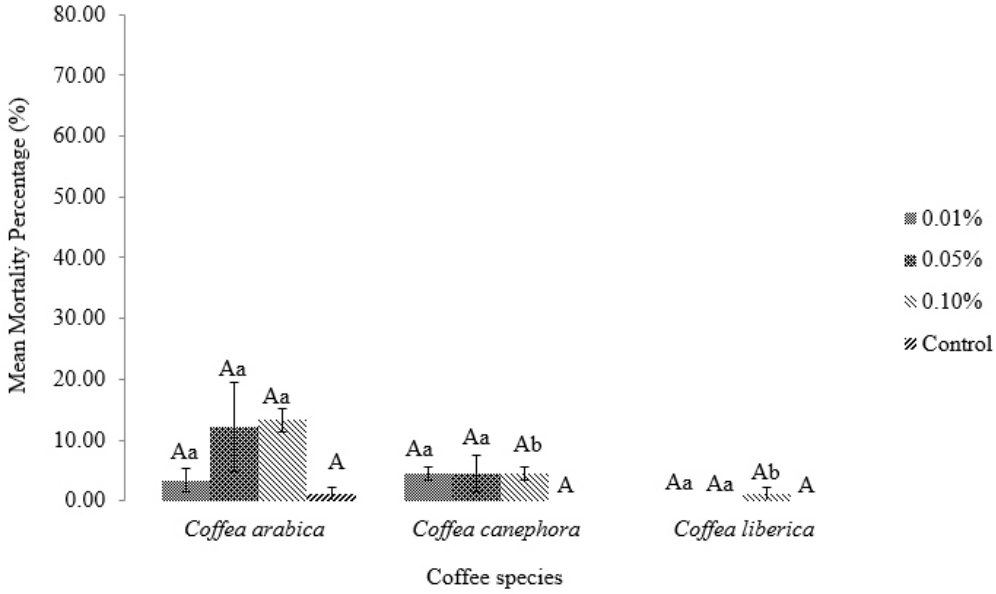

Figure 4. Mean mortality percentage of three different coffees against *P. megacephala* in Set II bioassay

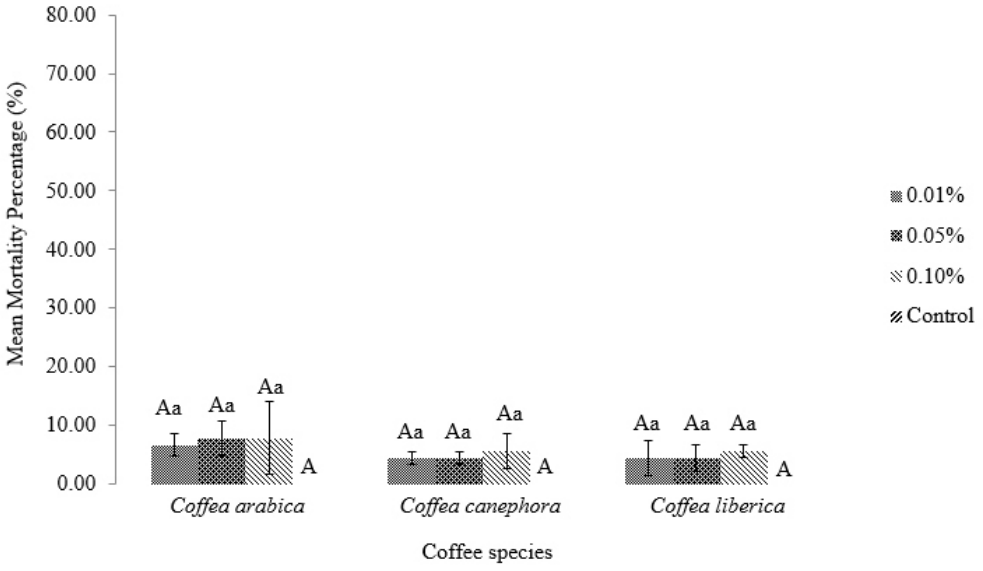

Figure 5. Mean mortality percentage of three different coffees against *M. pharaonis* in Set I bioassay

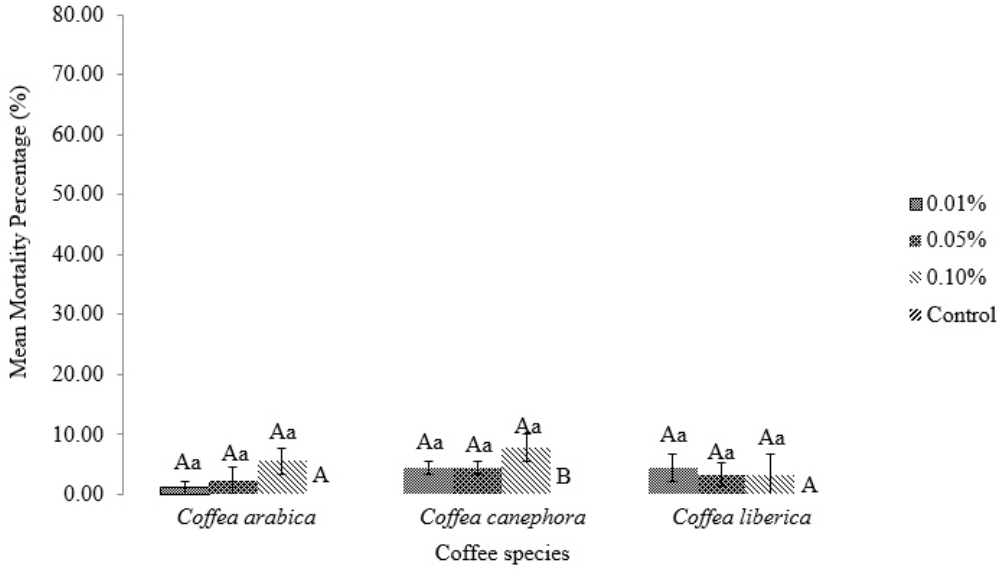

Figure 6. Mean mortality percentage of three different coffees against *M. pharaonis* in Set II bioassay

Effect of Coffee Extracts Impregnated in Gel Bait Towards the Feeding Behaviour of *T. indicum, P. megacephala and M. pharaonis*

In Set I bioassay, *P. megacephala* showed no significant difference (P>0.05) of their repellency behaviour at 30 min (P=0.416), 1 h (P=0.164), 2 h (P=0.382), 24 h (P=0.587), 48 h (P=0.056) and 72 h (P=0.229) between all concentrations of *C. arabica* and the control. At 4 h, the P values 0.038 displayed significant difference among the concentrations and control, at which 0.05% differed to 0.01% (P=0.043), 0.10% (P=0.043) and the control (P=0.046). At 8 h, the P value 0.028 (P<0.05) was a significant result; with 0.01% and 0.05% differed from both 0.10% and control (Table 3). For *C. canephora*, *P. megacephala* showed insignificant results for all time intervals except at 48 h (P=0.030, P<0.05); while there were no significant differences in *C. liberica* at all time intervals between the concentrations and control (Table 3).

In Set II bioassay, *C. arabica* indicated no significant differences of *P. megacephala* feeding behaviour at all time intervals with the exception of 48 h (P=0.023, P<0.05) and 72 h (P=0.025, P<0.05) between concentrations and control. At 48 h, 0.01% (P=0.05), 0.05% (P=0.05) and 0.10% (P=0.046) differed from the control. At 72 h, 0.05% and 0.10% had significant differences with 0.01% and the control (Table 4). *C. canephora* showed significant results at 30 min (P=0.043), 8 h (P=0.025), 24 h (P=0.013), 48 h (P=0.017) and 72 h (P=0.016) between concentrations and control. At 30 min, 8 h and 24 h, the results were significantly differed from the control but not among the three concentrations; while at 48 h and 72 h, the results displayed significant differences among concentrations and also with the control (Table 3). For *C. liberica*, only 48 h (P=0.024) and 72 h (P=0.024) showed significant results, at which they differed among the concentrations and with the control (Table 4).

For *M. pharaonis*, there were no significant results (P>0.05) of mean repellency percentage at all observed time intervals between the concentrations and control of all *Coffea* spp. in Set II bioassay (Table 6). On the other hand, in Set I bioassay, *C. liberica* displayed significant differences at 8 h (P=0.012), 24 h (P=0.013), 48 h (P=0.032) and 72 h (P=0.012); but there were no differences among the concentrations (Table 6).

In Set I bioassay, the mortality of *T. indicum* and *P. megacephala* increased with the increasing concentration (0.01%, 0.05% and 0.10%) of *C. arabica* and *C. canephora* but this trend was not shown in *C. liberica*. Instead, the results showed the lowest mortality at 0.05% of concentration for both the ant species, but still there were no significant differences (*T. indicum* P=0.054; *P. megacephala* P=0.114, P>0.05) among the three concentrations in *C. liberica* (Figures 1 and 5). At 0.05% of *C. liberica*, the mean repellency percentage for *P. megacephala* at 1 h, 2 h, 4 h, 8 h and 24 h are relatively higher as compared to that of 0.01% and 0.10%. These higher percentages indicated *P. megacephala* repelled more

Table 3

Mean mortality percentage and mean repellency percentage of three different coffees against P. megacephala *in Set I bioassay*

Coffee species	Concentration (%)	Mean Mortality Percentage (%)[1]	Mean Repellency Percentage (%)[2]							
			30min	1h	2h	4h	8h	24h	48h	72h
Coffea arabica	0.01%	11.11 ± 4.84Aa	84.45 ± 9.69C	88.89 ± 8.01C	84.44 ± 8.89C	-8.89 ± 4.44C	97.78 ± 2.22C	91.11 ± 5.88C	80.00 ± 7.70C	71.11 ± 8.01C
	0.05%	36.67 ± 6.67Aa	91.11 ± 2.22C	93.33 ± 3.85C	82.22 ± 8.01C	95.56 ± 4.44D	100.00 ± 0.00C	77.8 ± 9.69C	80.00 ± 3.85C	40.00 ± 34.21C
	0.10%	42.22 ± 4.01Aa	84.44 ± 15.56C	86.67 ± 6.67C	55.56 ± 14.57C	15.56 ± 11.11C	-53.33 ± 23.09D	68.89 ± 16.02C	42.22 ± 12.37C	77.78 ± 8.89C
	Control	4.45 ± 2.22B	48.89 ± 22.55C	48.89 ± 15.56C	53.33 ± 25.24C	-26.67 ± 17.64C	-60.00 ± 13.88D	20.00 ± 7.70C	31.11 ± 29.15C	24.22 ± 18.19C
Coffea canephora	0.01%	4.44 ± 1.11Aa	100.00 ± 0.00C	97.78 ± 2.22C	95.56 ± 4.44C	88.89 ± 8.01C	97.78 ± 2.22C	93.33 ± 6.67C	91.11 ± 8.89C	88.89 ± 4.44C
	0.05%	5.56 ± 2.94Ab	88.89 ± 8.01C	82.22 ± 11.11C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	-11.11 ± 5.88D	75.56 ± 8.01C
	0.10%	6.66 ± 3.33Ab	100.00 ± 0.00C	93.33 ± 3.85C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	93.33 ± 3.85C	82.22 ± 9.69C
	Control	0.00 ± 0.00A	88.89 ± 5.88C	82.22 ± 9.69C	93.33 ± 6.67C	82.22 ± 2.22C	100.00 ± 0.00C	82.22 ± 11.11C	-26.66 ± 6.67D	42.22 ± 15.56C

Table 3 *(Continue)*

Coffea liberica	**0.01%**	25.56 ± 9.87Aa	97.78 ± 2.22C	97.78 ± 2.22C	100.00 ± 0.00C	93.33 ± 6.67C	100.00 ± 0.00C	93.33 ± 3.85C	33.33 ± 26.94C	35.55 ± 18.99C
	0.05%	11.11 ± 2.22Ab	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	77.78 ± 8.89C	95.55 ± 2.22D
	0.10%	13.33 ± 3.33Ab	95.55 ± 2.22C	88.89 ± 11.11C	95.56 ± 4.44C	93.33 ± 3.85C	93.33 ± 3.85C	86.67 ± 10.18C	97.78 ± 2.22D	97.78 ± 2.22D
	Control	3.33 ± 1.93A	82.22 ± 11.11C	86.67 ± 10.18C	93.33 ± 3.85C	93.33 ± 0.00C	82.22 ± 5.88D	55.56 ± 19.75C	24.45 ± 13.52C	26.67 ± 30.06C

[1] Mean mortality percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: A & B (comparison of concentration within the same coffee species); a & b (comparison of coffee species within a concentration).
[2] Mean repellency percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: C, D & E (comparison of concentration within the same coffee species).

Table 4

Mean mortality percentage and mean repellency percentage of three different coffees against P. megacephala *in Set II bioassay*

Coffee species	Concentration (%)	Mean Mortality Percentage (%)[1]	Mean Repellency Percentage (%)[2]							
			30min	1h	2h	4h	8h	24h	48h	72h
Coffea arabica	0.01%	3.33 ± 1.93Aa	80.07 ± 13.81C	71.11 ± 14.57C	53.33 ± 3.85C	-31.11 ± 9.69C	-35.56 ± 27.84C	53.33 ± 11.55C	42.22 ± 25.04C	-42.22 ± 31.35D
	0.05%	12.22 ± 7.29Aa	77.78 ± 5.88C	80.00 ± 11.55C	62.22 ± 9.69C	-24.44 ± 15.55C	-46.66 ± 13.33C	86.67 ± 7.70C	77.78 ± 12.37C	42.22 ± 8.89C
	0.10%	13.33 ± 1.93Aa	84.45 ± 9.69C	68.89 ± 17.78C	57.78 ± 14.57C	73.33 ± 10.18C	42.22 ± 29.14C	68.89 ± 31.11C	95.55 ± 2.22C	80.00 ± 3.85C
	Control	1.11 ± 1.11A	57.78 ± 32.28C	60.00 ± 23.09C	48.89 ± 13.52C	-53.33 ± 37.12C	-62.22 ± 31.35C	46.67 ± 7.70C	-57.78 ± 21.20D	-57.78 ± 9.69D

Table 4 *(Continue)*

Coffea canephora	0.01%	4.44 ± 1.11Aa	91.11 ± 8.89C	60.00 ± 19.24C	91.11 ± 8.89C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	62.22 ± 8.01C
	0.05%	4.44 ± 2.94Aa	64.45 ± 9.69C	73.33 ± 17.64C	82.22 ± 8.89C	82.22 ± 11.76C	100.00 ± 0.00C	100.00 ± 0.00C	77.78 ± 5.88D	33.33 ± 7.70C
	0.10%	4.44 ± 1.11Ab	100.00 ± 0.00C	95.56 ± 4.44C	93.33 ± 6.67C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22D
	Control	0.00 ± 0.00A	48.89 ± 13.52D	37.78 ± 19.37C	68.89 ± 19.37C	55.56 ± 37.97C	71.11 ± 5.88D	57.78 ± 13.52D	-33.33 ± 16.78E	-51.11 ± 8.89E
Coffea liberica	0.01%	0.00 ± 0.00Aa	66.67 ± 11.55C	73.33 ± 10.19C	82.22 ± 8.01C	68.89 ± 27/84C	91.11 ± 5.88C	51.11 ± 19.75C	-53.33 ± 20.00C	-77.78 ± 5.88C
	0.05%	0.00 ± 0.00Aa	82.22 ± 8.01C	60.00 ± 30.55C	80.00 ± 10.18C	73.33 ± 23.41C	97.78 ± 2.22C	80.00 ± 20.00C	62.22 ± 5.88D	53.33 ± 3.85D
	0.10%	1.11 ± 1.11Ab	95.55 ± 2.22C	95.55 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22E	88.89 ± 11.11E
	Control	0.00 ± 0.00A	57.78 ± 35.55C	71.11 ± 22.22C	71.11 ± 25.63C	51.11 ± 36.38C	84.45 ± 9.69C	26.67 ± 10.19C	-55.55 ± 13.52C	-80.00 ± 10.18C

[1] Mean mortality percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: A & B (comparison of concentration within the same coffee species); a & b (comparison of coffee species within a concentration).

[2] Mean repellency percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: C, D & E (comparison of concentration within the same coffee species).

to the gel and resulted in lowest mortality. The overall results showed that the highest concentration, 0.10% of all three coffee species had the highest mortality against *T. indicum* (Figure 1). However, 0.01% of *C. liberica* showed the highest mortality against *P. megacephala* in the same set of gel bait as *P. megacephala* displayed the lowest mean repellency percentage at 48 h and 72 h (Table 3). For *M. pharaonis*, the mortality also showed a merely increasing trend from 0.01% to 0.10% but the differences were not much noticeable. For instances, 0.05% and 0.10% of *C. arabica* had the same mortality values; 0.01% and 0.05% of both *C. canephora* and *C. liberica* shared the similar mean mortality (Figure 5). This is because the mean repellency percentages for the three concentrations at all hours range from 95.55% to 100.00% (Table 5), indicating a very high repellency behaviour of *M. pharaonis*.

In Set II bioassay, there were increasing trends of *C. arabica* against *T. indicum*, *P. megacephala* and *M. pharaonis* with increasing concentrations (Figures 2 and 6). *C. canephora* and *C. liberica* showed that the lowest mortality on *T. indicum* was at 0.05% (Figure 2). On the other hand, *C. canephora* had the same mean mortality on *P. megacephala* at all concentrations (Figure 4) as the mean repellency percentage had no significant results from 30 min to 24 h (Table 4). The concentration of 0.01% *C. liberica* had a slightly higher mortality on *M. pharaonis* as the mean repellency percentages at 30 min, 1 h, 24 h and 72 h were lower than those of 0.05% and 0.10% (Figure 6 and Table 6). The lower repellency percentage indicated the more ants attracted to the gel and thus fed on the gel.

DISCUSSION

Residual spraying and baiting are common methods in controlling the pest ants. Baiting is considered a more effective measure as it is able to eliminate the entire colony through trophallaxis among the ants (Lee, 2000; Suiter et al., 1997). The uses of commercial and synthetic products have known to create certain issues such as environmental problem. Recent studies have revealed that plants act as potential insecticides, such as essential oil of *Pogostemon cablin* possess the insecticidal and repellence properties against the urban ants (Albuquerque et al., 2013). Plant secondary metabolites such as caffeine (1, 3, 7-trimethylxanthine) have pesticidal activity, anti-feeding properties and potential to be natural pesticide (Magalhães, Fernandes, Demuner, Picanco, & Guedes, 2010).

The overall results indicated *T. indicum* was the most susceptible species as it had the highest mortality among the three ant species. According to Lee, Lim and Yap (1996), the erratic movement of crazy ant allowed it to pick up more insecticidal materials and thus causing a higher mortality. This nature behaviour could also be observed in *T. indicum* at which they move rapidly and erratically, in turn leading to increased foraging activity and higher chance of picking up the toxicant, resulting in higher mortality (Lee et al., 1996). In addition, the very frequent

Table 5
Mean mortality percentage and mean repellency percentage of three different coffees against M. pharaonis *in Set I bioassay*

Coffee species	Concentration (%)	Mean Mortality Percentage (%)[1]	Mean Repellency Percentage (%)[2]							
			30min	1h	2h	4h	8h	24h	48h	72h
Coffea arabica	0.01%	6.67 ± 1.93Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	0.05%	7.78 ± 2.94Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C
	0.10%	7.78 ± 6.19Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	Control	0.00 ± 0.00A	97.78 ± 2.22C	95.56 ± 4.44C	95.55 ± 2.22C	93.33 ± 6.67C	97.78 ± 2.22C	91.11 ± 4.44C	86.67 ± 6.67C	91.11 ± 5.88C
Coffea canephora	0.01%	4.44 ± 1.11Aa	97.78 ± 2.22C	97.78 ± 2.22C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	95.55 ± 2.22C	95.56 ± 4.44C	100.00 ± 0.00C
	0.05%	4.44 ± 1.11Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	95.56 ± 4.44C	100.00 ± 0.00C	100.00 ± 0.00C
	0.10%	5.56 ± 5.09Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	Control	0.00 ± 0.00A	93.33 ± 6.67C	95.55 ± 2.22C	95.56 ± 4.44C	97.78 ± 2.22C	97.78 ± 2.22C	93.33 ± 3.85C	88.89 ± 5.88C	88.89 ± 5.88C

Table 5 *(Continue)*

Coffea liberica	0.01%	4.44 ± 2.94Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	0.05%	4.45 ± 2.22Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	0.10%	5.56 ± 1.11Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C
	Control	0.00 ± 0.00A	100.00 ± 0.00C	97.78 ± 2.22C	93.33 ± 6.67C	93.33 ± 6.67C	93.33 ± 0.00D	86.67 ± 3.85D	86.67 ± 3.85D	93.33 ± 0.00D

[1] Mean mortality percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: A & B (comparison of concentration within the same coffee species); a & b (comparison of coffee species within a concentration).
[2] Mean repellency percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: C, D & E (comparison of concentration within the same coffee species).

Table 6

Mean mortality percentage and mean repellency percentage of three different coffees against M. pharaonis *in Set II bioassay*

Coffee species	Concentration (%)	Mean Mortality Percentage (%)[1]	Mean Repellency Percentage (%)[2]							
			30min	1h	2h	4h	8h	24h	48h	72h
Coffea arabica	0.01%	1.11 ± 1.11Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	0.05%	2.22 ± 2.22Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	0.10%	5.55 ± 2.22Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	91.11 ± 8.89C
	Control	0.00 ± 0.00A	93.33 ± 6.67C	97.78 ± 2.22C	100.00 ± 0.00C	93.33 ± 6.67C	86.67 ± 13.33C	97.78 ± 2.22C	95.56 ± 4.44C	91.11 ± 8.89C

Table 6 *(Continue)*

Coffea canephora	0.01%	4.44 ± 1.93Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C
	0.05%	4.44 ± 1.93Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	93.33 ± 3.85C	97.78 ± 2.22C	100.00 ± 0.00C
	0.10%	7.78 ± 2.22Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	95.55 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C
	Control	0.00 ± 0.00B	100.00 ± 0.00C	95.56 ± 4.44C	100.00 ± 0.00C	97.78 ± 2.22C	95.56 ± 4.44C	88.89 ± 2.22C	82.22 ± 11.76C	84.45 ± 9.69C
Coffea liberica	0.01%	4.45 ± 2.22Aa	97.78 ± 2.22C	95.56 ± 4.44C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	100.00 ± 0.00C	95.55 ± 2.22C
	0.05%	3.33 ± 1.93Aa	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	97.78 ± 2.22C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	0.10%	3.33 ± 3.33Aa	100.00 ± 0.00C	97.78 ± 2.22C	97.78 ± 2.22C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C	100.00 ± 0.00C
	Control	0.00 ± 0.00A	100.00 ± 0.00C	97.78 ± 2.22C	93.33 ± 6.67C	100.00 ± 0.00C	95.56 ± 4.44C	95.56 ± 4.44C	93.33 ± 6.67C	95.55 ± 2.22C

[1] Mean mortality percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: A & B (comparison of concentration within the same coffee species); a & b (comparison of coffee species within a concentration).

[2] Mean repellency percentage followed by different letters within the same column are significant different by subjecting to Kruskal-Wallis H Test at $p<0.05$: C, D & E (comparison of concentration within the same coffee species.

self-grooming of *T. indicum* also contributed to its mortality. Ants perform self-grooming when they detect microbes or materials that endanger themselves (Hughes, Eilenberg, & Boomsma, 2002). They also tend to perform self-grooming for a longer time when encounter with more harmful microbes or materials (Morelos-Juárez, Walker, Lopes, & Hughes, 2010). During self-grooming, the ants may ingest the insecticidal-containing materials. Ants do perform allogrooming, a process of grooming towards other individuals by using their shovel-like mouthparts to remove potential harmful matters from the body surface (Wilson & Hölldobler, 2005). From the observation, *T. indicum* displayed a high frequency of allogrooming among them. This behaviour may cause them to accidentally ingest the toxicant on the body surfaces of other individuals, resulting in the highest mean mortality among the three ant species. On the contrary to *T. indicum*, *M. pharaonis* performed self-grooming and allogrooming less frequently, hence, they experienced the lowest mean mortality.

Trophallaxis is a process of exchanging regurgitated food among the colony members and it is very common to be observed among ants. It also allows the donors and recipients to gain information upon interaction. During trophallaxis, the ants share the food and even the insecticide-impregnated bait among themselves (Lee, 2000). The high frequency of trophallaxis displayed by *T. indicum* might lead to a higher chance for the other members to ingest the toxicant and result in a higher mortality value. Moreover, high performance of antennation among *T. indicum* might also contribute to its highest mortality. Based on the study by Hölldobler (1985) on ponerine ants, antennation functions in social greeting, recruitment and food solicitation. The touching the antennae with the others are known as tactile communication. Both trophallaxis and antennation play important role in food distribution and transmission (Hölldobler, 1985).

The results showed that the mortality increased with the increasing concentration (0.01%, 0.05% and 0.10%) for most but not all bioassays; hence, they were not concentration-dependent. This was in contrast with the previous study of effect of caffeine on tobacco hornworm larvae. The study indicated a dose-dependent effect at which higher concentration of caffeine lead to higher feeding and development inhibition of the larvae (Nathanson, 1984). In the present study, for instance, 0.01% of *C. liberica* showed the highest mortality against *P. megacephala* in Set I bioassay. This might be due to its lowest concentration and lowest mean repellency percentage at 48 h and 72 h indicating the ants were attracting the most to the bait and fed on it, thus causing the highest mortality. Other similar observations had also implied the higher repellency percentages result in lowest mortality of *P. megacephala* in Set I bioassay and *T. indicum* in Set II bioassay at 0.05% of *C. liberica*. Hence, it can be concluded that the repellency percentage is associated with the ant mortality. In addition, Set II *C. canephora* bioassay of the big-headed ants showed almost same or the same mean mortality at all concentrations (Figure 4) although there were fluctuations

of the mean repellency percentage at 48 h and 72 h. This occurrence can be explained by the delay action of the toxicant in the gel bait, which is one of the important characteristics of the gel (Knight & Rust, 1991).

The big-headed ants, *P. megacephala* are known as one of the worst and highly invasive ant species. They have a better ability to discover and exploit food resources than other native ant species (Callan & Majer, 2009). This observation was similar to the results in this present study; at which *P. megacephala* showed higher attraction behaviour towards the bait as they are able to locate the food attractant better. Though they were attracted mostly to the bait when compared to the other two ant species, their ant mortality is not the highest. According to Cokendolpher and Francke, (1985), the ant body size affects their desiccation rate. Ants with smaller body size possess larger surface area to volume ratios tend to desiccate faster (Cokendolpher & Francke, 1985). Both minor and major workers of *P. megacephala* that are larger in size experience a lower desiccation rate and hence lower mortality. However, the smaller size of Pharaoh ant did not show a higher mortality value even though their sizes are much smaller than the big-headed ants. The high repellency behaviour observed in *M. pharaonis* caused the lowest mean mortality. This might be due to the Pharaoh ants showing higher degree of repellency towards the water and sugar solution incorporated in the bait; as according to Fowler et al. (1993), *M. pharaonis* prefer high protein food. In addition, the slow and inactive movement of Pharaoh ant may also explain their lowest mortality.

Almost all Set I bioassays showed higher mortality as compared to Set II bioassay for all three ant species. *T. indicum* displayed feeding preference towards carbohydrate foods but there was no specific preference of carbohydrate foods (Chong & Lee, 2006). According to Lee (2000), most household ants were attracted most to 20% to 30% of sucrose solution. As a proven, *T. indicum* preferred Set I gel bait that consisted of 20% sugar solution and assumed to feed more on the bait, thus resulting in a higher mortality as compared to that of Set II gel bait. Albeit there are food preferences in different ant species, ants do display higher preference towards the sugar solution when compared to water as the carbohydrate providing energy to them (David & Venkatesha, 2013). This explained that most of the results showing higher mortality in Set I bioassay.

Among the three coffee species, *C. arabica* showed the highest ant mortality on *T. indicum*, *P. megacephala* and *M. pharaonis* at almost all concentrations in both Set I and Set II bioassays. According to Itoyama and Bicudo (1992), caffeine reduces the mating frequency, egg-laying capacity, fertility and longevity of *Drosophila prosaltans* (Diptera: Drosophilidae). Further research showed that caffeine suppressed the feeding activity of flies and beetles (Pedronel, Casanova, Ortiz, Henao, & Pelaez, 2007). However, the data obtained was in contrast with those previous studies. From our record on GC-MS analysis (unpublished data), *C.*

arabica contained the lowest composition percentage of caffeine (25.31%) when compared to *C. canephora* (44.70%) and *C. liberica* (47.30%). *C. arabica* occupied the lowest composition percentage of caffeine but it had the highest mortality against the ants. This has suggested caffeine may not be the main compound of *Coffea* spp. to cause ant mortality. From the study on bumblebees, nectar toxins such as caffeine, quinine, nicotine, amygdalin and grayanotoxin do not impede the pollination activity of bumblebees (Tiedeken, Stout, Stevenson, & Wright, 2014). Same to the research on honeybees, low concentrations of the caffeine tend to enhance their visitation frequency to the solution (Hagler & Buchmann, 1993). Moreover, bees show poor acuity and weak sensitivity of detecting plant toxins in sucrose solution (Tiedeken et al., 2014). As both ants and bees are eusocial insects and from the same order of Hymenoptera, it is possible to assume that ants possess the same nature with bees, indicating that caffeine is not the cause of causing mortality.

C. arabica contained undecane, hexadecanamide and tetradecanamide which could not be found in the other two coffee species. Undecane is a volatile hydrocarbon compound and also an alarm pheromone that can be found in the ants (Lenz, Krasnec, & Breed, 2013). Regnier and Wilson (1969) reported that a minute amount of undecane caused some ant species move rapidly. Undecane had also shown attraction and excitement in the workers of the crazy ants (Witte, Attygalle, & Meinwald, 2007). Another outcome had demonstrated this alarm pheromone allow recruitment of workers to the disturbance region. The high volatility of undecane improves the rate of spreading of this compound to the surrounding, increasing the activity of the ants (Lenz et al., 2013). Based on these evidences, undecane is the possible compound in coffee that attracts ants, increases their movement and activities. This can also be proven that the repellency percentages in most *C. arabica* bioassays are lower. However, the possibility of undecane to be the compound in coffee that causes ant mortality is yet to be known.

Many studies revealed the effectiveness of coffee in controlling insects. Caffeine was known to block the larval development of *Aedes aegypti* (Diptera: Culicidae) and cause lethal effect. The effect is dose-dependent as the higher the concentration of the caffeine, the faster the blockade of larval development (Laranja et al., 2003). Caffeine also impedes the oviposition activity and drags the appearance of developmental stages in the life cycle of the tea shot-hole borer beetle, *Euwallacea* (= *Xyleborus*) *fornicates* (Coleoptera: Scolytidae). Nevertheless, there was no observed lethal outcome on the beetle (Hewavitharanage et al., 1999). From a recent study on leaf-cutting ants *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) by Miyashira, Tanigushi, Gugliotta and Santos (2012), caffeine had no significant effect on their survival but decreased the growth rate of the mutualistic fungus of leaf-cutting ants. The fungal growth rate decreased with the increasing

concentration of caffeine. The mutualistic fungus acts as the only food source for the immature stages of leaf-cutting ants at which they require glycogen-rich diet for development. The adults obtained nutrients from the decomposition process of plant tissue by the fungus while the fungus gained benefit from the competition free circumstances with other microorganisms. The symbiotic relationship between the leaf-cutting ants and fungus had demonstrated the ants might be associated with caffeine toxicity to the fungus but not affected directly by the caffeine (Miyashira et al., 2012). According to this study, again, it can be concluded caffeine has little or no direct lethal effect on the ants, therefore matching the data obtained in this research.

Nevertheless, the chemical composition of the coffee compounds may vary dependind on the roasting temperature and time. For instance, the coffee roasted at higher temperatures for a shorter period display higher acidity, more soluble solids and a different volatile profile while comparing with coffee that roasted at a lower temperature with longer period of time (Farah, 2012). Therefore, it is very crucial to have standardized and constant roasting temperature and time to obtain a reliable chemical composition percentage of the compounds while comparing the coffee species.

Basically, the higher concentration of caffeine had led to a higher repellency. Honey bees were less likely to consume the sucrose solution with high dose of caffeine (Mustard, Dews, Brugato, Dey, & Wright, 2012). Nonetheless, this phenomenon was not observed in this study. The concentrations, 0.01%, 0.05% and 0.10% used were based on the study of Miyashira et al. (2012). The repellency behaviours of *T. indicum* and *M. pharaonis* towards the three concentrations were similar. While *P. megacephala* showed some degree of significant results at only 8, 24, 48 and 72 h, at which higher repellency was observed at higher concentration. This is probably due to the highest concentration, 0.10% used in this research was considered low to deter the feeding of ants. It was shown that 0.30% to 10% of caffeine suppressed the feeding activity and growth of tobacco hornworm larvae, *Manduca sexta* (Lepidoptera: Sphingidae) (Nathanson, 1984). From the results obtained and according to these previous studies, coffee that consists of various volatile and non-volatile compounds has the potential to act as a repellent for ants. Minor compounds may act as potent synergists to increase the impact of major compounds. Therefore, future research can be performed to reinforce the caffeine repellency effect on ants by using a higher concentration.

CONCLUSION

In conclusion, all the ant species displayed slightly higher mortality in the bioassay with bait containing sugar attractant. Coffee with low concentration was not effective in killing the household ants because the mortalities obtained after three days did not exceed 50%. Therefore, future researches to test on the lethal effect of coffee can be studied by using higher concentration. Moreover, the potential of coffee to be formulated as ant repellent cannot be ruled out as the overall results showed a great

extent of repellency towards the baits. The natural behaviour of the ant had also contributed to their mortalities. *T. indicum* with higher frequency of trophallaxis, self-grooming, allogrooming and antennation had the highest mortality among the three ant species tested. A further study could be conducted to test the coffee effect on the ant colony instead of only on the ant workers. Furthermore, *C. arabica* had the best impact on the ant mortality. Hence, a focus study on the effect of this coffee species on ants should be carried out in the future.

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REFERENCES

Ab Majid, A. H., Dieng, H., Ellias, S. S., Sabtu, F. S., Abd Rahim, A. H., & Satho, T. (2018). Olfactory behavior and response of household ants (Hymenoptera) to different types of coffee odor: A coffee-based bait development prospect. *Journal of Asia-Pacific Entomology*, *21*(1), 46–51.

Abdullah, F., Subramanian, P., Ibrahim, H., Malek, S. N. A., Lee, G. S., & Hong, S. L. (2015). Chemical composition, antifeedant, repellent, and toxicity activities of the rhizomes of galangal, *Alpinia galanga* against Asian subterranean termites, *Coptotermes gestroi* and *coptotermes curvignathus* (Isoptera: Rhinotermitidae). *Journal of Insect Science*, *15*(1), 7.

Albuquerque, E. L. D., Lima, J. K. A., Souza, F. H. O., Silva, I. M. A., Santos, A. A., Araújo, A. P. A., … Bacci, L. (2013). Insecticidal and repellence activity of the essential oil of *Pogostemon cablin* against urban ants species. *Acta Tropica*, *127*(3), 181–186.

Beatson, S. (1972). Pharaoh's ants as pathogen vectors in hospitals. *The Lancet*, *299*(7747), 425–427.

Callan, S. K., & Majer, J. D. (2009). Impacts of an incursion of African big-headed ants, *Pheidole megacephala* (Fabricius), in urban bushland in Perth, Western Australia. *Pacific Conservation Biology*, *15*(2), 102–115.

Campos-Farinha, A. E. (2005). Urban Pest Ants of Brazil (Hymenoptera: Formicidae). In C.Y. Lee & W. H. Robinson (Eds.), *Proceedings of the Fifth International Conference on Urban Pests* (pp. 1–4). Suntec, Singapore: Perniagaan Ph'ng @ P&Y Design Network, Malaysia.

Chong, K. F., & Lee, C. Y. (2006). Food preferences and foraging activity of field populations of a pest ant, *Tapinoma indicum* (Hymenoptera: Formicidae). *Sociobiology*, *48*(3), 875–883.

Cokendolpher, J. C., & Francke, O. F. (1985). Temperature preferences of four species of fire ants (Hymenoptera: Formicidae: Solenopsis). *Psyche (New York)*, *92*(1), 91–101.

David, A. I., & Venkatesha, M. G. (2013). Attraction of household ants (Hymenoptera: Formicidae) to various food sources in different seasons. *Journal of Entomology*, *10*(2), 66–75.

Eow, A. G. H., Chong, A. S. C., & Lee, C. Y. (2004). Colonial growth dynamics of tropical urban pest ants, *Monomorium pharaonis*, *M. floricola* and *M. destructor* (Hymenoptera: Formicidae). *Sociobiology*, *44*(2), 365–377.

Farah, A. (2012). Coffee constituents. *Coffee: Emerging Health Effects and Disease Prevention*, *1*, 22–58.

Fowler, H. G., Filho, F. A., & Bueno, O. C. (1993). Seasonal space usage by the introduced Pharaoh's ant, *Monomorium pharaonis* (L.) (Hym., Formicidae), in institutional settings in Brazil and its relation to other structural ant species. *Journal of Applied Entomology*, *115*(1–5), 416–419.

Gooch, H. (1999). Baiting remains the treatment of choice. *Pest Control*, *67*(9), 40–43.

Hagler, J. R., & Buchmann, S. L. (1993). Honey bee (Hymenoptera: Apidae) foraging responses to phenolic-rich nectars. *Journal of the Kansas Entomological Society*, *66*(2), 223–230.

Hewavitharanage, P., Karunaratne, S., & Kumar, N. S. (1999). Effect of caffeine on shot-hole borer beetle (*Xyleborus fornicatus*) of tea (*Camellia sinensis*). *Phytochemistry*, *51*(1), 35–41.

Higure, Y., & Nohmi, M. (2002). Repetitive application of caffeine sensitizes caffeine-induced Ca^{2+} release in bullfrog sympathetic ganglion neurons. *Brain Research, 954*(1), 141-150.

Hölldobler, B. (1985). Liquid food transmission and antennation signals in ponerine ants. *Israel Journal of Entomology, 19*, 89-99.

Hughes, W. O., Eilenberg, J., & Boomsma, J. J. (2002). Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. *Proceedings of the Royal Society of London B: Biological Sciences, 269*(1502), 1811–1819.

Itoyama, M. M., & Bicudo, H. E. M. C. (1992). Effects of caffeine on fecundity, egg laying capacity, development time and longevity in *Drosophila prosaltans*. *Revista Brasilliensis de Genetica*, *15*(2), 303–321.

Jaramillo, J., Borgemeister, C., & Baker, P. (2006). Coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): searching for sustainable control strategies. *Bulletin of Entomological Research*, *96*(3), 223–233.

Jenkins, M. (2001). Battling a common enemy. *Pest Control*, *69*(9), S10-S12, S16, S27.

Kaminski, L. (2000). Ants are still tops, for now. *Pest Control*, *68*(9), S9-S11.

Klotz, J. H., Greenberg, L., Shorey, H. H., & Williams, D. F. (1997). Alternative control strategies for ants around homes. *Journal of Agricultural Entomology*, *14*(3), 249–257.

Knight, R. L., & Rust, M. K. (1991). Efficacy of formulated baits for control of Argentine ant (Hymenoptera: Formicidae). *Journal of Economic Entomology*, *84*(2), 510–514.

Laranja, A. T., Manzatto, A. J., & de Campos Bicudo, H. E. M. (2003). Effects of caffeine and used coffee grounds on biological features of *Aedes aegypti* (Diptera, Culicidae) and their possible use in alternative control. *Genetics and Molecular Biology*, *26*(4), 419–429.

Lee, C. Y. (2000). Performance of hydramethylnon- and fipronil-based containerized baits against household ants in residential premises. *Tropical Biomedicine*, *17*(1), 45–48.

Lee, C. Y., Lim, C. Y., & Yap, H. H. (1996). Contact toxicity of bendiocarb against three species of Malaysian household ants (Hymenoptera: Formicidae). *Journal of Biosciences*, *7*(1), 79–82.

Lenz, E. L., Krasnec, M. O., & Breed, M. D. (2013). Identification of undecane as an alarm pheromone of the ant *Formica argentea*. *Journal of Insect Behavior*, *26*(1), 101–108.

Lucky, A. (2009). Urban ants of North America and Europe: Identification, biology, and management-edited by John Klotz, Lauren Hansen, Reiner Pospischil and Michael Rust. *Systematic Entomology*, *34*(2), 406–407.

Magalhaes, S. T. V., Fernandes, F. L., Demuner, A. J., Picanço, M. C., & Guedes, R. N. C. (2010). Leaf Alkaloids, phenolics, and coffee resistance to the

leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Journal of Economic Entomology, 103*(4), 1438-1443.

Marer, P., & Flint, M. (1991). *Residential, industrial and institutional pest control* (2nd ed.). Oakland, California: University of California, Statewide Integrated Pest Management Program, Agriculture and Natural Resources.

Mgbemena, I. C., Ebe, T., Nnadozie, A.I., & Ekeanyanwu, K.K. (2015). Repellent activities of the methanolic leaf extracts of *Moringa oleifera* and *Stachytarpheta indica* against *Aedes aegypti* mosquito. *Journal of Pharmacy and Biological Sciences, 10*(4), 77-81.

Miyashira, C. H., Tanigushi, D. G., Gugliotta, A. M., & Santos, D. Y. (2012). Influence of caffeine on the survival of leaf-cutting ants *Atta sexdens rubropilosa* and *in vitro* growth of their mutualistic fungus. *Pest Management Science*, *68*(6), 935–940.

Morelos-Juárez, C., Walker, T. N., Lopes, J. F. S., & Hughes, W. O. H. (2010). Ant farmers practice proactive personal hygiene to protect their fungus crop. *Current Biology*, *20*(13), R553-R554.

Mustard, J. A., Dews, L., Brugato, A., Dey, K., & Wright, G. A. (2012). Consumption of an acute dose of caffeine reduces acquisition but not memory in the honey bee. *Behavioural Brain Research*, *232*(1), 217–224.

Na, J. P. S., & Lee, C.Y. (2001). Identification key to common urban pest ants in Malaysia. *Tropical Biomedicine, 18*(1), 1-17.

Nathanson, J. (1984). Caffeine and related methylxanthines: possible naturally occurring pesticides. *Science*, *226*(4671), 184–187.

Pedronel, A., Casanova, H., Ortiz, C., Henao, B., & Pelaez, C. (2007). Insecticidal activicity of caffeine aqueous solutions and caffeine oleate emulsions against *Drosophila melanogaster* and *Hypothenemus hampei*. *Journal of Agricultural and Food Chemistry, 55*(17), 6918-6922.

Regnier, F. E., & Wilson, E. O. (1969). The alarm-defence system of the ant *Lasius alienus*. *Journal of Insect Physiology*, *15*(5), 893–898.

Satho, T., Dieng, H., Ahmad, M. H. I., Ellias, S. B., Hassan, A. A., Abang, F., … Nolasco-Hipolito, C. (2015). Coffee and its waste repel gravid *Aedes albopictus* females and inhibit the development of their embryos. *Parasites and Vectors*, *8*(1), 272.

Smith, S. E., McElhatton, P. R., & Sullivan, F. M. (1987). Effects of administering caffeine to pregnant rats either as a single daily dose or as divided doses four times a day. *Food and Chemical Toxicology*, *25*(2), 125–133.

Suiter, D., Wu, D., & Bennett, G. (1997). The evolution of ant control. *Pest Control*, *65*, 46–51.

Tiedeken, E. J., Stout, J. C., Stevenson, P. C., & Wright, G. A. (2014). Bumblebees are not deterred by ecologically relevant concentrations of nectar toxins. *Journal of Experimental Biology*, *217*(9), 1620–1625.

Williams, D. F. (1989). An improved artificial nest for laboratory rearing of the imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Florida Entomologist*, *72*(4), 705–707.

Wilson, E. O., & Holldobler, B. (2005). The rise of the ants: A phylogenetic and ecological explanation. *Proceedings of the National Academy of Sciences*, *102*(21), 7411–7414.

Witte, V., Attygalle, A. B., & Meinwald, J. (2007). Complex chemical communication in the crazy ant *Paratrechina longicornis* Latreille (Hymenoptera: Formicidae). *Chemoecology*, *17*(1), 57–62.