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Plant Derived Pesticides (*Citrus hystrix* DC, *Mentha* x *piperita* L., *Ocimum basilicum* L.) in Controlling Household Ants (*Tapinoma indicum* (F.), *Pheidole megacephala* (F.), *Monomorium pharaonis* (L.)) (Hymenoptera: Formicidae)

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ABSTRACT

The pest ants-related predicament is increasing in urban area and becoming a problem for most of the citizen. The most common approach for pest ants' control is the application of various insecticide spray. However, the long-term insect pest control must not be dependent on synthetic insecticides with many negative effects. Repellence and insecticidal effects from plants, *Citrus hystrix*, *Mentha piperita* and *Ocimum basilicum* have been reputed for different type of insect pests. The combination of an effective botanical insecticide and gel bait is ideal for the treatment of insect pests. In this study, the repellence and insecticidal effects of plant extracts obtained from three plant species with various concentrations (3×10^5, 5×10^5, 7×10^5, and 1×10^6 ppm) were against common urban pest ants, *Tapinoma indicum*, *Pheidole megacephala* and *Monomorium pharaonis* using repellence, insecticidal and gel bait bioassays. The three plant extracts usually repel ants with the efficacy being dependent on the ants and irrespective of plant species. Repellence and mortality of ants were negatively related to the higher percentage (100% = fully repelled; -100% = fully attracted; 0% = neither repelled nor attracted) of being repelled, the lower the death will be. Moreover, the optimal doses that make the fastest mortality of ants are

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not consistent between the insecticidal and gel bait bioassays.

Keywords: Bio-pesticide, control, household ants, plant extract

INTRODUCTION

Ants are the third most abundant household pests in urban areas, after mosquitoes and cockroaches, as they always foraging in groups have raised annoyances (Lee, 2002). Household pest ants foraging in kitchen area may result in foods contamination and the contaminated foods may not be suitable for consumption. Moreover, ants can serve as carrier of pathogens for several diseases and raised alert of health problems for some people which are more sensitive to insects' bite (Lee & Tan, 2004).

Synthetic or man-made insecticides are currently most common method used for pest ant's management as it is fast and effective. However, it is also necessary to keep in mind the side effects of the continuous use of synthetic or chemical insecticides including the growing of insect pests that are resistant to man-made insecticide, environmental pollution, possibly harmful health of the operators and causing unnecessary dwindling of species (Hebling et al., 2000; Khater, 2012). The efficacy of insecticide spray is also limited considering this method could not reach the heart of the colony which is the queen of the ant's population and only affects the foraging ants which just make up a section of the whole colony (Hanna et al., 2015). Moreover, in comparison to the laboratory, the efficacy of biological control using natural predators in the field is unstable and sometimes, ineffective, considering the constant changing environmental circumstances (Castaño-Quintana et al., 2013).

Bait integrated with toxic active ingredient is currently used to deal with problems of purging worker ants. Toxic bait is highly efficient in colony removal by indulge the pest ants with toxic attractant and utilise the common behaviour of ants, trophallaxis of which allow the ants to disperse the toxic active ingredient throughout the colony, to achieve whole colony elimination. Nevertheless, active ingredient commonly integrated in commercial bait are synthetic insecticides. Thus, botanical insecticides that can achieve the same efficiency as synthetic insecticides could be used to replace them. Botanical-based insecticides are safer, eco-friendly, species-specific, decompose quickly and have very low occurrence of insect pest resistance (Khater 2012; Pavela et al., 2010).

Plants are common in producing secondary metabolites and the toxic properties of these production have been evaluated since ancient times to use against various household insect pests (Adeyemi, 2010; George et al., 2000). Plant extracts and essential oil are becoming increasingly important to replace the synthetic active ingredient in the bait, to minimize synthetic pesticide dependency. In the present study, repellence as well as insecticidal effect of kaffir lime (*Citrux hystrix*), peppermint

(*Mentha piperita*), and basil (*Ocimum basilicum*) were evaluated against the common household pest ants including ghost ants (*Tapinoma indicum*), big-headed ants (*Pheidole megacephala*) and pharaoh ants (*Monomorium pharaonis*). The extracted crude ingredients of plants were also infused into the gel and the performance of gel bait with botanical insecticides were evaluated.

MATERIALS AND METHODS

Plants Extraction

The plant's part used for extraction of kaffir lime, peppermint, and basil were dried leaves using Soxhlet extraction (Handa, 2008). About 30-40g of dried plant leaves were placed into the thimble and a flask contained methanol (about 250-260 mL) as extracting solvent was placed below the thimble. The bottle of methanol was heated, and the vapours contacted with plant materials. The soluble active compounds from the plant material were then transferred into the vaporised methanol. The vapours then condensed, dripped back into thimble, and flowed back into the flask. The flask of methanol was slowly be replaced by methanol that contained the crude extracts of plants. The procedure of the extraction lasted approximately 5-6 hours. The solvent that contained the extract was dried for 1-2 days. About 1-3 g of plant's crude could be obtained and the extract was ready to use.

Prepared solution (concentration calculated / formula):

Parts per million =
$$\frac{\text{grams of solute (g)}}{\text{grams of solution (ml)}} \text{ X } 1{,}000{,}000$$

Ants Collection

In the present study, three type of ants, ghost ants, big-headed ants, and pharaoh ants were collected with live traps from places around the School of Biological Sciences, Universiti Sains Malaysia. The ants were identified based on the descriptions by Klotz et al. (2008).

Bioassays

Repellence Bioassay. Different Concentrations (3×10^5, 5×10^5, 7×10^5 and 1×10^6 ppm) of crude extract was prepared. Whatman® No.1 filter paper (90 mm) was splatted in half with one half dipped the solution of crude extracts of various concentrations and the other half was leaving clean. Control was filter paper dipped with distilled water (Figure 1). Thirty ants were used for each treatment of each ant species. Each test was replicated three times. Number of ants at several time intervals including 1 hour after the experiment set up, 3 hours, 6 hours, 12 hours, and every 24 hours up to 3 days of both sides were determined. The behaviour or response as well as the activities of ants toward the tested plant extracts was observed during the bioassay. The repellence percentage was calculated with the following formula (Abdullah et al., 2015):

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

where, NC = Number of ants on the non-treated side and NT = Number of ants on the treated side.

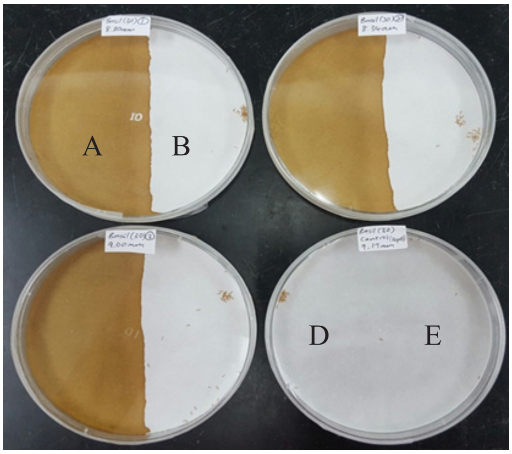

Figure 1. Repellence bioassay of 3 x 10^5 ppm of basil (treated side A and untreated side B) with replicate one, two, three (start from the left to right and bottom left) and control plates (bottom right) (side D treated with distilled water and untreated side E)

Insecticidal Bioassay. Four concentrations of crude extract (3 x 10^5, 5 x 10^5, 7 x 10 ^{5} and 1 x 10^6 ppm) of each tested plant extract were applied on the ants' pronotum with 3 replications. Ants inoculated with distilled water used as control. Collected ant samples were rather aggressive with high mobility, thus, the whole collection tube which contained the ant samples were placed inside the refrigerator for 3-4 minutes to reduce the ants' activity or mobility. The extract solutions (0.1 µL) were then pipetted on the pronotum region. Following the bioassay, mortality of ants was assessed by counting the number of perished samples at time intervals including 1 hour after the experiment set up, 3 hours, 6 hours, 12 hours and every 24 hours and up to 3 days. The ant was considered no longer alive if the individuals remained motionless for 1-2 minutes and no respond given when the body was touched with brush. The behavioural or response as well as any activities showed by the ants toward the tested plant extracts during the bioassay were recorded.

Gel Bait Bioassay. The plant extracts were diluted with 20% sugar solution to several concentrations (3 x 10^5, 5 x 10^5, 7 x 10^5 and 1 x 10^6 ppm). Ferti-plant jelly (Fertiland Trading Co., Malaysia) was then dipped into the plant extract sugar solution, and the jelly absorb and expanded within the solution about 5 hours. The expanded jelly was then measured about 1 g and used as mimic gel bait (Figure 2). Jelly dipped with pure sugar solution was used as control. Repellence effect of the prepared gel as well as the mortality of ants due to after contact with the gel were evaluated at several time intervals including 1 hour after the experiment set up, 3 hours, 6 hours, 12 hours, and every 24 hours up to 3 days. The ant was considered no longer alive if the particular individuals remained motionless for 1-2 minutes and no respond given when the body was touched with brush. The behavioural response as well as any activities showed by the ants toward the tested plant extracts during the bioassay were recorded.

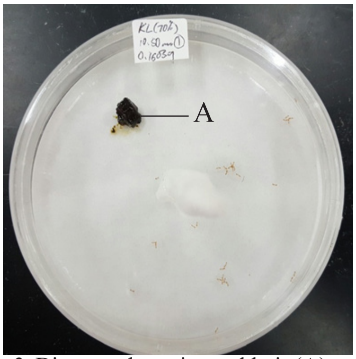

Figure 2. Bioassay by using gel bait (A)

Statistical Analysis

Results from the bioassay on repellence were recorded as percentage of which (100% = fully repelled; -100% = fully attracted; 0% = neither repelled nor attracted). For insecticide and gel bait bioassays, percentage of mortality of each tested ant species were presented. We used Pearson Correlation to demonstrate how the repellence efficacy of the crude extracts relate to ant's mortality (Hinton et al., 2014). The lethal time (LT_{50}, LT_{90}) was calculated with Probit analysis for all plant tested extracts (Akcay, 2013). Analysis of Variance (ANOVA) and significant differences among the various concentrations of the extracts were analysed by Tukey's test ($p < 0.05$) using IBM SPSS Statistic version 22.

RESULTS

Repellence Bioassay

Percentage of Repellence of Tested Plant Extracts with Various Concentrations. Based on Table 1, ghost ants had shown highly repelled from four concentrations of all tested extracts at each time interval. The percentage of repellence (PR) without negative sign had shown that ghost ants were staved off the tested plant extracts, regardless of concentrations (PR = 100% = entirely repelled, PR = 0 = neither attracted nor repelled from the tested extracts, PR = -100% = entirely attracted). Nevertheless, reaction of the tested ant species against the repellence effect showed by each plant extracts was varied. Table 2 presented that big-headed ants was not staved off by all the tested plant extracts and even constantly moving to the side that contain the crude extracts. On the other hand, pharaoh ants were repelled by various concentrations of each plant extract (Table 3) and also more likely to stay idle. Self-grooming of ghost ants could be perceived at high frequency, particularly after the ants were moving out from treated side. Big-headed ants' major workers are most likely surrounded by their minor workers, while individual's aggregation was regularly shown in pharaoh ants.

Mortality of Ants after Contact the Plant Extracts in Repellence Bioassay. There was ant's mortality in repellence bioassay. Ants could have taken the active ingredient of the plant extracts when they moved the side covered with extract solution of which lethal to the tested ants. In the treatments of peppermint (1×10^6 ppm) and basil (3×10^5 ppm), big headed ants had the highest death rate of which full mortality (100%) was observed within 48 hours (Table 5) considering the ants were only little repulsed by the tested plant extracts. The result was also suggesting that big-headed ants were more vulnerable compared to ghost ants and pharaoh ants. Ghost ants and pharaoh ants showed similar rate of mortality (Tables 4 and 6). Efficacy of repellence was negatively associated with the mortality of ants.

Table 1
Percentage of repellence of three plant extracts with various concentrations against Tapinoma indicum

Treatment	Concentration (ppm)	Percentage of repellence (%)						
		1 h	3 h	6 h	12 h	24 h	48 h	72 h
Citrus hystrix	3 x 10^5	84.44±4.443a	84.44±12.372a	88.89±11.110a	88.89±8.013a	75.56±15.555a	66.66±17.638a	57.78±18.189a
	5 x 10^5	97.78±2.223a	100.00±0.000a	100.00±0.000a	95.55±2.223a	93.33±0.000a	77.78±8.890a	57.78±15.553a
	7 x 10^5	100.00±0.000a	95.55±2.223a	97.78±2.223a	88.89±5.879a	71.11±5.879a	42.22±5.879a	48.89±12.372a
	1 x 10^6	88.89±4.443a	100.00±0.000a	93.33±3.848a	100.00±0.000a	93.33±6.667a	82.22±5.879a	60.00±10.183a
Mentha piperita	3 x 10^5	86.67±3.848a	82.22±8.890a	84.45±9.686a	80.00±10.183a	82.22±5.879a	73.33±3.848a	55.55±17.777a
	5 x 10^5	88.89±11.110a	91.11±5.879a	93.33±3.848a	93.33±0.000a	91.11±2.220a	84.44±5.880ab	48.89±14.573a
	7 x 10^5	95.55±2.223a	93.33±3.848a	97.78±2.223a	91.11±5.879a	80.00±7.696a	57.78±9.686a	46.67±3.848a
	1 x 10^6	100.00±0.000a	95.55±2.223a	95.56±4.443a	97.78±2.223a	42.22±2.223ab	42.22±2.223a	17.78±14.573a
Ocimum basilicum	3 x 10^5	80.00±13.879a	97.78±2.223a	100.00±0.000a	93.33±3.848a	100.00±0.000a	97.78±2.223ab	95.55±2.223ab
	5 x 10^5	86.67±3.848a	95.55±2.223a	82.22±2.223a	97.78±2.223a	91.11±4.443a	93.33±3.848ab	86.67±3.848ab
	7 x 10^5	91.11±4.443a	93.33±3.848a	93.33±3.848a	91.11±5.879a	86.67±7.699a	75.55±13.518a	62.22±14.573a
	1 x 10^6	91.11±2.223a	95.55±2.223a	100.00±0.000a	95.55±2.223a	97.78±2.223a	84.45±2.223ab	91.11±2.220ab

* Means followed by the same letter in each column are not significantly different ($P > 0.05$)

Table 2
Percentage of repellence of three plant extracts with various concentrations against Pheidole megacephala

Treatment	Concentration (ppm)	Percentage of repellence (%)						
		1 h	3 h	6 h	12 h	24 h	48 h	72 h
Citrus hystrix	3 x 10^5	73.33±0.000ab	80.00±13.879a	86.67±7.699a	75.55±13.518a	62.22±2.223a	33.33±26.667a	31.11±28.890a
	5 x 10^5	17.78±13.518a	73.33±6.667a	57.78±11.759a	44.44±19.749a	8.89±23.200a	2.22±19.371a	-4.44±14.571a
	7 x 10^5	46.67±13.876a	64.44±8.013a	73.33±3.848a	40.00±10.183a	24.44±11.113a	17.78±22.555a	17.78±22.555a
	1 x 10^6	86.67±0.000ab	86.67±6.667a	64.44±5.880a	60.00±0.000a	62.22±5.879a	53.33±3.848a	53.33±3.848a
Mentha piperita	3 x 10^5	-4.44 ±15.555a	60.00±7.696a	62.22±8.890a	53.33±13.878a	15.55±17.777a	22.22±2.223a	22.22±2.223a
	5 x 10^5	-15.56 ±24.745b	-22.22 ±13.518b	46.67±7.699a	-4.45±21.199ab	11.11±23.518a	13.33±34.210a	28.89±34.712a
	7 x 10^5	46.67±10.182ab	55.55±13.518a	75.56±8.013a	53.33±7.699a	55.56±8.013a	42.22±8.890a	42.22±9.686a
	1 x 10^6	53.33±3.848a	66.67±10.182a	73.33±6.667a	55.55±11.759a	26.67±7.7699a	26.67±7.699a	24.44±8.013a

Table 2 *(Continued)*

Treatment	Concentration (ppm)	Percentage of repellence (%)						
		1 h	3 h	6 h	12 h	24 h	48 h	72 h
Ocimum basilicum	3×10^5	40.00±6.667ab	51.11±8.890a	66.66±13.333a	48.89±5.879a	40.00±3.851a	40.00±3.851a	40.00±3.851a
	5×10^5	62.22±14.573ab	55.56±4.443a	46.67±13.333a	68.89±8.890a	4.44±17.356a	15.56±12.372a	31.11±8.890a
	7×10^5	55.56±11.113a	80.00±10.183a	82.22±2.223a	71.11±9.688a	64.44±8.013a	40.00±6.667a	36.67±6.938a
	1×10^6	62.22±9.686ab	68.90±5.878a	64.44±5.880a	57.78±9.685a	46.66±6.667a	40.00±7.696a	44.44±5.880a

* Means followed by the same letter in each column are not significantly different (P > 0.05)

Table 3

Percentage of repellence of three plant extracts with various concentrations against Monomorium pharaonis

Treatment	Concentration (ppm)	Percentage of repellence (%)						
		1 h	3 h	6 h	12 h	24 h	48 h	72 h
Citrus hystrix	3×10^5	88.89±4.443a	91.11±4.443a	95.56±4.443a	84.44±12.372a	31.11±27.306ab	33.33±6.667a	33.33±6.667ab
	5×10^5	100.00±0.000ab	100.00±0.000a	97.78±2.223a	100.00±0.000a	97.78±2.223a	88.89±5.879a	88.89±5.879a
	7×10^5	97.78±2.223a	95.55±2.223a	97.78±2.223a	100.00±0.000a	93.33±3.848a	64.44±17.356a	71.11±22.552a
	1×10^6	100.00±0.000ab	97.78±2.223a	100.00±0.000a	100.00±0.000a	100.00±0.000a	100.00±0.000ab	95.55±2.223a

Table 3 *(Continued)*

Treatment	Concentration (ppm)	Percentage of repellence (%)						
		1 h	3 h	6 h	12 h	24 h	48 h	72 h
Mentha piperita	3×10^5	97.78±2.223a	97.78±2.223a	97.78±2.223a	100.00±0.000a	100.00±0.000a	95.56±4.443ab	95.56±4.443a
	5×10^5	91.11±2.220a	95.55±2.223a	91.11±4.443a	100.00±0.000a	95.55±2.223a	75.55±9.686a	60.00±15.398a
	7×10^5	97.78±2.223a	100.00±0.000a	97.78±2.223a	95.55±2.223a	95.55±2.223a	88.89±4.443a	75.55±2.223a
	1×10^6	100.00±0.000ab	100.00±0.000a	97.78±2.223a	93.33±6.667a	86.67±7.699a	68.89±17.356a	62.22±16.024a
Ocimum basilicum	3×10^5	100.00±0.000ab	100.00±0.000a	95.56±4.443a	100.00±0.000a	100.00±0.000a	95.55±2.223ab	95.56±4.443a
	5×10^5	100.00±0.000ab	95.55±2.223a	100.00±0.000a	88.89±8.013a	73.33±13.878ab	48.89±26.201a	40.00±17.638a
	7×10^5	97.78±2.223a	100.00±0.000a	97.78±2.223a	100.00±0.000a	100.00±0.000a	100.00±0.000ab	95.55±2.223a
	1×10^6	97.78±2.223a	97.78±2.223a	100.00±0.000a	97.78±2.223a	88.89±5.879a	77.78±15.553a	0.000±0.000ab

* Means followed by the same letter in each column are not significantly different ($P > 0.05$)

Table 4
Mortality of Tapinoma indicum *after contacted with various concentrations of three plant extracts in repellence bioassay*

Treatment	Concentration (ppm)	Mortality (%)						
		1 h	3 h	6 h	12 h	24 h	48 h	72 h
Citrus hystrix	3 x 10^5	-	2.22±2.223a	4.45±2.223a	12.22±5.879a	20.00±3.333a	27.78±7.777a	37.78±4.005a
	5 x 10^5	-	0.00±0.000a	3.33±1.925a	6.67±1.925a	15.56±2.940a	33.33±5.774a	56.67±13.876ab
	7 x 10^5	-	1.11±1.110a	3.33±1.925a	8.89±4.008a	15.56±2.940a	71.11±17.248ab	90.00±10.000b
	1 x 10^6	-	0.00±0.000a	0.00±0.000a	5.55±2.223a	17.78±4.005a	57.78±8.011ab	98.89±1.110b
Mentha piperita	3 x 10^5	-	0.00±0.000a	1.11±1.110a	1.11±1.110a	3.33±1.925a	3.33±1.925a	4.45±2.223a
	5 x 10^5	-	1.11±1.110a	1.11±1.110a	3.33±3.333a	6.67±5.093a	13.33±8.389a	24.45±11.277a
	7 x 10^5	-	0.00±0.000a	2.22±2.223a	5.56±1.113a	13.33±1.925a	24.44±6.761a	47.78±10.599ab
	1 x 10^6	-	1.11±1.110a	1.11±1.110a	4.44±2.940a	15.56±5.557a	63.33±10.713ab	93.33±3.333b
Ocimum basilicum	3 x 10^5	-	0.00±0.000a	2.22±1.110a	2.22±1.110a	2.22±1.110a	3.33±1.925a	3.33±1.925a
	5 x 10^5	-	1.11±1.110a	3.33±3.333a	6.67±1.925a	6.67±1.925a	12.22±1.110a	20.00±3.333a
	7 x 10^5	-	5.55±2.223ab	11.11±1.110ab	17.78±6.187ab	22.22±9.095ab	32.22±12.223a	47.78±11.110ab
	1 x 10^6	-	0.00±0.000a	0.00±0.000a	1.11±1.110a	1.11±1.110a	6.66±3.333a	17.78±1.110a
Control	-	-	0.00±0.000a	0.00±0.000a	0.00±0.000a	0.00±0.000a	0.00±0.000a	0.00±0.000a

* Means followed by the same letter in each column are not significantly different ($P > 0.05$)
* '-' No test conducted due to no mortality

Table 5

Mortality of Pheidole megacephala *after contacted with various concentrations of three plant extracts in repellence bioassay*

Treatment	Concentration (ppm)	Mortality (%) 1 h	3 h	6 h	12 h	24 h	48 h	72 h
Citrus hystrix	3×10^5	6.67±6.667a	20.00±13.879a	30.00±20.817a	58.89±27.845ab	66.67±31.683a	75.56±22.799a	82.22±16.140a
	5×10^5	2.22±2.223a	3.33±3.333a	14.45±7.779a	42.22±20.215a	83.33±12.019a	95.56±4.443a	98.89±1.110a
	7×10^5	0.00±0.000a	12.22±5.553a	20.00±5.774a	48.89±10.599a	86.67±3.333a	98.89±1.110a	100.00±0.000a
	1×10^6	1.11±1.110a	25.56±12.372a	52.22±12.223ab	78.89±2.940ab	93.33±1.925a	95.55±2.223a	97.78±2.223a
Mentha piperita	3×10^5	0.00±0.000a	0.00±0.000a	0.00±0.000a	17.78±4.447a	50.00±6.938ab	92.22±2.939a	96.67±0.000a
	5×10^5	0.00±0.000a	0.00±0.000a	0.00±0.000a	14.45±4.005a	44.44±4.841ab	86.67±5.774a	95.55±2.223a
	7×10^5	0.00±0.000a	6.67±1.925a	26.67±5.093a	52.22±6.758a	83.34±6.667a	91.11±5.557a	100.00±0.000a
	1×10^6	0.00±0.000a	2.22±2.223a	17.78±2.223a	56.67±6.938a	95.55±2.223a	100.00±0.000a	100.00±0.000a
Ocimum basilicum	3×10^5	0.00±0.000a	3.33±0.000a	18.89±6.758a	60.00±11.547ab	97.78±1.110a	100.00±0.000a	100.00±0.000a
	5×10^5	0.00±0.000a	0.00±0.000a	3.33±1.925a	21.11±7.776a	44.45±11.759ab	85.56±9.492a	97.78±1.110a
	7×10^5	1.11±1.110a	7.78±2.939a	23.33±3.333a	37.78±1.110a	62.22±4.005a	94.45±2.223a	98.89±1.110a
	1×10^6	0.00±0.000a	2.22±2.223a	20.00±3.851a	46.67±7.699a	93.33±1.925a	94.44±1.113a	97.78±2.223a
Control	-	0.00±0.000a	0.00±0.000a	0.00±0.000a	0.00±0.000a	0.00±0.000b	0.00±0.000b	0.00±0.000b

* Means followed by the same letter in each column are not significantly different ($P > 0.05$)

Table 6

Mortality of Monomorium pharaonis *after contacted with various concentrations of three plant extracts in repellence bioassay*

Treatment	Concentration (ppm)	Mortality (%)						
		1 h	3 h	6 h	12 h	24 h	48 h	72 h
Citrus hystrix	3×10^5	-	2.22±2.223a	2.22±2.223a	4.44±4.443a	42.22±16.024a	100.00±0.000a	100.00±0.000a
	5×10^5	-	0.00±0.000a	1.11±1.110a	2.22±1.110a	4.44±1.113ab	6.67±1.925b	7.78±2.939b
	7×10^5	-	0.00±0.000a	0.00±0.000a	0.00±0.000a	5.56±2.940ab	42.22±14.573ab	87.78±12.223a
	1×10^6	-	0.00±0.000a	1.11±1.110a	2.22±1.110a	5.55±4.005ab	5.55±4.005b	15.55±6.187b
Mentha piperita	3×10^5	-	0.00±0.000a	1.11±1.110a	1.11±1.110a	1.11±1.110ab	1.11±1.110b	3.33±1.925b
	5×10^5	-	2.22±1.110a	2.22±1.110a	10.00±1.923a	27.78±11.600ab	75.56±22.799ab	84.44±15.557a
	7×10^5	-	0.00±0.000a	2.22±1.110a	4.44±1.113a	6.67±1.925ab	13.33±3.848b	26.67±8.389b
	1×10^6	-	1.11±1.110a	4.44±4.443a	10.00±8.390a	21.11±8.678ab	32.22±7.777ab	47.78±10.941ab
Ocimum basilicum	3×10^5	-	0.00±0.000a	0.00±0.000a	0.00±0.000a	0.00±0.000b	0.00±0.000b	1.11±1.110b
	5×10^5	-	0.00±0.000a	0.00±0.000a	0.00±0.000a	22.22±11.277ab	65.56±32.792ab	78.89±21.110ab
	7×10^5	-	0.00±0.000a	0.00±0.000a	1.11±1.110a	1.11±1.110b	1.11±1.110b	5.55±2.223b
	1×10^6	-	0.00±0.000a	0.00±0.000a	1.11±1.110a	4.44±2.934b	28.89±7.776ab	70.00±15.275ab
Control	-	-	0.00±0.000a	0.00±0.000a	0.00±0.000a	0.00±0.000b	0.00±0.000b	0.00±0.000b

* Means followed by the same letter in each column are not significantly different (P > 0.05)
* '-' No test conducted due to no mortality

Repellence Efficacy Correlates with Ants Mortality. Pearson correlation was conducted to demonstrate how the repellence efficacy related with mortality of ants. Tables 7, 8 and 9 showed that the efficacy of repellence was negatively associated with ant mortality, extract that high in repellence effect, the less likely of causing ants' mortality. Nevertheless, positive Pearson correlation coefficients were shown in some treatments of which the ants were more attracted to the treated side but low mortality.

Table 7

Repellence efficacy of plant extracts correlate with Tapinoma indicum *mortality*

Treatment	Concentration (ppm)	r
Citrus hystrix	3 x 10^5	-0.726**
	5 x 10^5	-0.913**
	7 x 10^5	-0.917**
	1 x 10^6	-0.817**
Mentha piperita	3 x 10^5	-0.190
	5 x 10^5	-0.424
	7 x 10^5	-0.865**
	1 x 10^6	-0.832**
Ocimum basilicum	3 x 10^5	0.288
	5 x 10^5	-0.206
	7 x 10^5	-0.870**
	1 x 10^6	-0.383

** Correlation is significant at P < 0.01

Table 8

Repellence efficacy of plant extracts correlate with Pheidole megacephala *mortality*

Treatment	Concentration (ppm)	r
Citrus hystrix	3 x 10^5	-0.273
	5 x 10^5	-0.721**
	7 x 10^5	-0.678**
	1 x 10^6	-0.851**
Mentha piperita	3 x 10^5	-0.350
	5 x 10^5	0.161
	7 x 10^5	-0.216
	1 x 10^6	-0.776**
Ocimum basilicum	3 x 10^5	-0.369
	5 x 10^5	-0.483*
	7 x 10^5	-0.595**
	1 x 10^6	-0.624**

** Correlation is significant at P < 0.01
* Correlation is significant at P < 0.05

Table 9

Repellence efficacy of plant extracts with Monomorium pharaonis *mortality*

Treatment	Concentration (ppm)	r
Citrus hystrix	3 x 10^5	-0.754**
	5 x 10^5	-0.809**
	7 x 10^5	-0.708**
	1 x 10^6	-0.275
Mentha piperita	3 x 10^5	-0.220
	5 x 10^5	-0.543*
	7 x 10^5	-0.897**
	1 x 10^6	-0.910**
Ocimum basilicum	3 x 10^5	0.102
	5 x 10^5	-0.963**
	7 x 10^5	-0.416
	1 x 10^6	-0.921**

** Correlation is significant at P < 0.01
* Correlation is significant at P < 0.05

Insecticidal Bioassay

Probit Analysis. Lethal time of causing 50% and 90% of ants' mortality was determined using Probit analysis. The analysis was important in evaluate the efficacy of each of the concentration of tested plant extracts in killing tested ants' species as the shorter time needed in causing 50% and 90% death of the population, indicating the more effective of the plants being used as botanical insecticide. Each tested plant with various concentrations showed varied insecticide effect toward the tested ant species (Tables 10, 11 and 12). The findings showed that the efficiency of plant extracts in killing tested ants was not dose-based, where low concentration could lead to higher ant's mortality than the high concentration.

Kaffir lime extract with concentration, 5 x 10^5 ppm was the most effective treatment for ghost ants with LT_{50} of about 3 hours and 4 minutes (Table 10). On the contrary, big-headed ants reach 50% of mortality within 1 hour and 47 minutes using basil crude extract with concentration, 1 x 10^6 ppm (Table 11). Peppermint extract with concentration, 1 x 10^6 ppm was most usefully used against pharaoh ants with LT_{50} of about 7 hours and 48 minutes (Table 12).

In general, for ghost ants, about 3-9 hours were needed for the tested population to reach 50% mortality in all tested plant extracts (Table 10) while pharaoh ants were about 10 hours (Table 12). Big-headed ants have the lowest LT_{50} of which about 1-7 hours (Table 11). The results again support the previous hypothesis that the big-headed ants are vulnerable and more susceptible than others toward contaminant and toxicant.

Table 10

Time needed to reach 50% and 90% lethal of Tapinoma indicum *inoculated with various concentrations of tested plant extracts*

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		X^2	df
			Lower	Upper		Lower	Upper		
Citrus hystrix	3 x 10^5	4.89	3.67	6.38	10.32	7.63	18.73	17.66a	5
	5 x 10^5	3.08	2.23	4.06	9.32	6.69	15.96	12.59a	5
	7 x 10^5	6.38	5.08	7.94	15.76	12.01	24.18	10.85a	5
	1 x 10^6	3.71	3.19	4.27	12.99	10.83	16.25	5.99a	5
Mentha piperita	3 x 10^5	5.97	5.43	6.55	11.53	10.13	13.65	3.72a	5
	5 x 10^5	4.29	3.77	4.86	12.32	10.45	15.12	7.61a	5
	7 x 10^5	5.67	4.53	7.00	15.79	12.10	23.27	8.93a	5
	1 x 10^6	3.69	2.73	4.82	10.67	7.73	17.93	12.65a	5

Table 10 *(Continued)*

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		X^2	df
			Lower	Upper		Lower	Upper		
Ocimum basilicum	3×10^5	9.78	6.21	15.54	24.75	15.57	73.53	38.12a	5
	5×10^5	5.31	3.54	7.72	15.13	9.95	33.97	24.58a	5
	7×10^5	3.67	3.20	4.17	10.70	9.05	13.18	3.53a	5
	1×10^6	5.60	4.04	7.63	13.74	9.67	26.78	19.89a	5

* Means followed by the same letter in each column are not significantly different ($P > 0.05$)

Table 11

Time needed to reach 50% and 90% lethal of Pheidole megacephala *inoculated with various concentrations of tested plant extracts*

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		χ^2	*df*
			Lower	Upper		Lower	Upper		
Citrus hystrix	3×10^5	7.38	6.47	8.39	24.48	20.57	30.27	3.48a	5
	5×10^5	3.00	2.39	3.68	7.41	5.79	10.74	8.47b	5
	7×10^5	2.49	1.84	3.20	6.60	4.93	10.44	11.27b	5
	1×10^6	3.48	1.39	6.43	9.01	5.16	58.84	57.06b	5
Mentha piperita	3×10^5	6.56	4.78	8.68	37.13	23.64	63.36	8.74a	5
	5×10^5	3.72	2.81	4.78	8.82	6.60	14.30	13.56b	5
	7×10^5	3.19	2.44	4.06	6.94	5.27	11.11	13.25b	5
	1×10^6	3.06	2.48	3.71	7.03	5.57	9.97	8.20b	5
Ocimum basilicum	3×10^5	2.78	2.45	3.12	6.50	5.61	7.83	2.26b	5
	5×10^5	2.82	2.51	3.15	5.80	5.09	6.85	4.17b	5
	7×10^5	2.77	1.07	4.83	6.06	3.70	36.08	53.81b	5
	1×10^6	1.79	1.58	2.01	3.65	3.17	4.36	3.46b	5

* Means followed by the same letter in each column are not significantly different ($P > 0.05$)

Table 12

Time needed to reach 50% and 90% lethal of Monomorium pharaonis *inoculated with various concentrations of tested plant extracts*

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		X^2	df
			Lower	Upper		Lower	Upper		
Citrus hystrix	3×10^5	12.05	7.45	20.46	26.42	16.63	98.37	48.27a	5
	5×10^5	10.84	8.61	13.75	22.64	17.18	36.52	14.86a	5
	7×10^5	14.75	10.87	20.12	33.31	23.69	62.61	22.68a	5
	1×10^6	13.98	10.52	18.77	28.03	20.50	52.02	22.50a	5

Table 12 *(Continued)*

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		X^2	df
			Lower	Upper		Lower	Upper		
Mentha piperita	3×10^5	13.73	10.68	17.86	27.43	20.53	47.03	18.50a	5
	5×10^5	14.40	10.98	18.97	26.50	19.91	48.30	22.10a	5
	7×10^5	17.59	12.24	25.39	42.01	28.42	92.57	29.13a	5
	1×10^6	7.80	4.30	13.77	23.17	13.27	95.37	47.66a	5
Ocimum basilicum	3×10^5	20.07	11.84	35.35	49.01	29.47	204.36	52.51a	5
	5×10^5	14.21	10.04	20.27	34.07	23.24	71.91	26.95a	5
	7×10^5	14.99	13.71	16.39	27.63	24.50	32.26	2.14a	5
	1×10^6	11.81	3.98	35.16	30.66	15.26	1473.16	105.01b	5

* Means followed by the same letter in each column are not significantly different (P > 0.05)

Bioassay using Gel Bait

Probit Analysis. Gel mixed with various concentrations of plant extracts exhibited slow mortality effect, indicating more time is needed for the ants to be killed considering indirect and delayed contact of ants with the active ingredient within the crude extracts. However, the mortality trend of the tested ant species remained the same as big-headed ants was the first to succumb with LT_{50} of about 10-29 hours (Table 14), followed by ghost ants (14-48 hours) (Table 13) and lastly, pharaoh ants (29-56 hours) (Table 15).

Based on Tables 13-15, the ants' mortality was not dose-related as in some of the low concentration treatments having shorter time in leading to ants' mortality. According to Table 13, basil extract with concentration, 3×10^5 ppm was the most effective treatment against ghost ants with LT_{50} of about 14 hours and 40 minutes while the same plant species with concentration, 5×10^5 ppm was most useful in against big-headed ants (LT_{50}, 10 hours and 51 minutes) (Table 14). Pharaoh ants were most susceptible toward kaffir lime with concentration, 7×10^5 ppm (LT_{50}, 29 hours and 12 minutes) (Table 15).

Table 13

Time needed to reach 50% and 90% lethal of Tapinoma indicum *after contacted with various concentrations of tested plant extracts integrated into gel*

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		X^2	df
			Lower	Upper		Lower	Upper		
Citrus hystrix	3×10^5	47.98	29.23	134.75	136.54	70.23	-	44.48a	5
	5×10^5	33.10	-	-	64.00	-	-	-	5
	7×10^5	36.34	24.38	57.36	82.19	55.36	280.10	36.40a	5
	1×10^6	26.02	8.03	104.30	50.25	28.43	-	112.08a	5

Table 13 *(Continued)*

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		X^2	df
			Lower	Upper		Lower	Upper		
Mentha piperita	3 x 10^5	31.70	-	-	54.53	-	-	-	5
	5 x 10^5	30.73	22.96	41.25	46.10	35.75	90.71	31.60a	5
	7 x 10^5	28.61	24.74	33.12	43.58	36.99	57.56	10.18a	5
	1 x 10^6	25.37	-	-	51.23	-	-	-	5
Ocimum basilicum	3 x 10^5	14.67	9.00	24.66	26.18	17.74	117.60	50.78b	5
	5 x 10^5	15.28	9.93	23.84	39.74	25.16	108.06	36.33b	5
	7 x 10^5	27.04	-	-	41.94	-	-	-	5
	1 x 10^6	25.99	3.96	339.61	45.88	26.87	-	115.64c	5

* '-' Fiducial limits could not be generated

* Same column with same letter is not significantly different (P > 0.05)

Table 14

Time needed to reach 50% and 90% lethal of Pheidole megacephala *after contacted with various* concentrations of tested plant extracts integrated into gel

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		χ^2	*df*
			Lower	Upper		Lower	Upper		
Citrus hystrix	3 x 10^5	29.10	19.05	49.31	43.44	31.67	207.31	50.29a	5
	5 x 10^5	24.11	18.42	31.73	42.61	32.26	76.63	24.00a	5
	7 x 10^5	19.15	13.50	27.63	32.83	23.82	80.37	35.13a	5
	1 x 10^6	16.93	13.40	21.56	25.33	20.23	43.70	22.48a	5
Mentha piperita	3 x 10^5	29.16	22.46	38.85	43.53	34.04	85.54	27.05a	5
	5 x 10^5	17.33	16.10	18.64	24.81	22.69	27.97	7.75b	5
	7 x 10^5	18.57	14.47	23.89	34.04	26.03	56.93	19.58a	5
	1 x 10^6	25.36	-	-	48.59	-	-	871.79	5
Ocimum basilicum	3 x 10^5	15.29	-	-	24.97	-	-	1083.45	5
	5 x 10^5	10.85	7.65	15.85	21.96	15.20	52.54	31.49c	5
	7 x 10^5	10.91	8.50	14.19	21.44	16.07	37.36	18.38c	5
	1 x 10^6	11.85	9.87	14.30	23.68	18.86	33.96	10.29c	5

* '-' Fiducial limits could not be generated

* Same column with same letter is not significantly different (P > 0.05)

Table 15

Time needed to reach 50% and 90% lethal of Monomorium pharaonis *after contacted with various concentrations of tested plant extracts integrated into gel*

Treatment	Concentration (ppm)	LT_{50}	95% Confidence limits (hours)		LT_{90}	95% Confidence limits (hours)		X^2	df
			Lower	Upper		Lower	Upper		
Citrus hystrix	3 x 10^5	49.36	34.07	66.42	77.86	60.21	256.67	30.30a	5
	5 x 10^5	32.06	-	-	49.38	-	-	-	5
	7 x 10^5	29.20	19.39	43.43	57.25	39.50	149.75	40.52a	5
	1 x 10^6	31.32	-	-	53.95	-	-	927.81	5
Mentha piperita	3 x 10^5	56.02	42.21	88.88	113.69	76.83	483.61	23.26a	5
	5 x 10^5	39.64	17.10	288.34	104.91	50.94	-	86.19b	5
	7 x 10^5	40.42	15.71	69.29	62.39	44.28	985.64	70.74b	5
	1 x 10^6	32.90	20.06	51.10	51.38	36.93	175.84	55.84b	5
Ocimum basilicum	3 x 10^5	38.71	18.36	127.86	89.72	49.16	-	77.88b	5
	5 x 10^5	29.46	22.81	38.75	44.01	34.58	82.53	26.35b	5
	7 x 10^5	38.35	22.19	58.79	61.59	44.30	242.59	56.66b	5
	1 x 10^6	39.68	36.84	42.53	61.14	56.10	68.33	5.35a	5

* '-' Fiducial limits could not be generated

* Same column with same letter is not significantly different (P > 0.05)

DISCUSSION

Repellence and Insecticide Efficiency of Three Plants

In repellence bioassay, all three tested plant extracts of various concentrations showed repellence effect with all tested ants (ghost ants, big-headed ants and pharaoh ants). Ants already showed repellence against to the lowest concentration which was 3 x 10^5 ppm from each tested plant extract let alone the higher concentrations (5 x 10^5, 7 x 10^5 and 1 x 10^6 ppm) tested.

Ants species that tested in the present study avoid contact with the crude extract (treated side), the tested plants species are commonly produced secondary metabolites such as linalool, citronellol, and β-citronellol by *C. hystrix* (Loh et al., 2011; Nor, 1999), menthone by *M. piperita* as well as trans-anethole, estragole and linalool in *O. basilicum* (Chang et al., 2009), which have repellence (Kumar et al., 2011; Tawatsin et al., 2001) and insecticidal effect (Bakkali et al., 2008; Loh et al., 2011). Ants can also be repelled as they perceive the pungent smell emitted from crude extracts through their olfactory system (Ab Majid et al., 2018).

Ghost ants and pharaoh ants were highly repelled from the plant extracts and had slow mortality. However, big-headed ants were generally attracted to the treated side, followed by high mortality in all treatments. Big-headed ants are considered as predator species or invasive ants in natural environment (Hoffmann & O'Connor, 2004) and intrinsically tend to constantly extend their colony by producing a large amount of worker ants to compete with other species (Dejean et al., 2007). The aggressive characteristic of this species might be the reason for big-headed ants was less repelled from all tested plant crude extracts. Nonetheless, as this species tends to produce many individual ants in order to be dominant on a particular area, the ants tend to have lower survival rate (Fournier et al., 2009). This phenomenon was shown in all bioassays in the present study of which big-headed ants took lesser time to reach mortality. Therefore, it is assumed that non-aggressive ant species such as ghost ants and pharaoh ants have reduced mortality rates to compensate for their competition weakness in natural environment.

Ant's Mortality

Mortality of ants could be observed throughout the study. This may be due to the ants' intrinsic behaviour including self- and allo-grooming when confronted with contaminant and the contacted active ingredient (Hughes et al., 2002) was accidentally ingested except in the insecticidal bioassay of which the active ingredient was unavoidable. Self-grooming is the individual self-cleaning behaviour while allo-grooming are group-level cleaning behaviours that remove the contaminants from another individual of which commonly happened in ant society considering the ants are capable of detecting the presence of contaminant as well as the risk of infection that occurred either on their own self or on their nestmates (Bos et al., 2012; Morelos-Juárez et al., 2010; Walker & Hughes, 2009). Thus, the frequency of self- and allo-grooming of all tested ants were high as shown in the present study due to this behaviour as a way to remove the contaminant. Although allo-grooming could decrease the contamination surface of ants, this behaviour was not performed as frequent as self-grooming throughout the experiment considering that the contaminated ants were tending to exclude themselves from their broods and perished in isolation reduce the chances of its broods getting contaminated as well (Bos et al., 2012).

Self-grooming can even happen before contamination takes place or when a detrimental substance is detected. This occurrence may indicate that self-cleaning behaviour is more likely intrinsic than a simple pathogens reaction (Morelos-Juárez et al., 2010). However, grooming could be a useful behaviour to be used in insect pest control.

Potential of Botanical Insecticide that used in Toxic Bait

The insecticidal effects of kaffir lime, peppermint and basil showed in the present study were similar and not significantly different from one another ($p < 0.05$). In these three different plant species, similar chemical components of the secondary metabolites could be the primary reason for similarity in insecticide effect. Thus, the different rate of mortality among the tested ants may due to their different susceptibility level toward the plant extracts. Nevertheless, the supplication of plant extracts directly or indirectly does increase the rate of mortality of all the tested ant species as the ant samples within the control treatment remain alive and active.

With its inclusion into a gel, the potential of kaffir lime, peppermint and basil to be used as botanical insecticide was evaluated. The repellence effect of all the tested plant species was offset by adding sugar as an attractant with insecticidal effect was not impaired. Furthermore, the movement of ants was not affected immediately and there was a minor mortality in the first three hours of each treatment which could meet the requirement of the delayed action of insecticide required in gel bait (Williams et al., 2001). The phenomenon of ' bait-shyness ' (Greaves, 1989) was not observed throughout the experiment and ants were willing to go near to the toxicant contained gel. Results of the gel bait bioassays showed 100% of mortality can be attained within 2-3 days for most replicates in different treatments.

CONCLUSION

The tested ants become very susceptible increased rate of mortality after contact with the solution from all the tested plant extracts. Different plant extracts were effective in treated specific species in different bioassays. In insecticidal bioassay, kaffir lime (5 x 10^5 ppm) was the most efficient in control ghost ants while in gel bait bioassay, basil (3 x 10^5 ppm) causing the shortest $LT_{50.}$ Big-headed ants were most effectively controlled by basil with concentration, 1 x 10^6 ppm in insecticidal bioassay while in gel bait bioassay, higher concentration (5 x 10^5 ppm) of the same species was more useful. Peppermint (1 x 10^6 ppm) was the most effective in purging pharaoh ants in insecticidal bioassay while kaffir lime (7 x 10^5 ppm) was the most useful in gel bait bioassay.

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CONFLICT OF INTEREST STATEMENT

The researchers declare that there is no conflict of interest.

REFERENCES

Ab Majid, A. H., Dieng, H., Ellias, S. S., Sabtu, F. S., Rahim, A. H., & Satho, T. (2018). Olfactory behaviour 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.

Adeyemi, M. H. (2010). The potential of secondary metabolites in plant material as deterrents against insect pests: A review. *African Journal of Pure and Applied Chemistry*, *4*(11), 243-246.

Akcay, A. (2013). The calculation of LD50 using probit analysis. *The Federation of American Societies for Experimental Biology Journal*, *27*(1), 1217-1228.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology*, *46*(2), 446-475.

Bos, N., Lefevre, T., Jensen, A. B., & d'Ettorre, P. (2012). Sick ants become unsociable. *Journal of Evolutionary Biology*, *25*(2), 342-351.

Castaño-Quintana, K., Montoya-Lerma, J., & Giraldo-Echeverri, C. (2013). Toxicity of foliage extracts of *Tithonia diversifolia* (Asteraceae) on *Atta cephalotes* (Hymenoptera: Myrmicinae) workers. *Industrial Crops and Products*, *44*, 391-395.

Chang, C. L., Cho, I. K., & Li, Q. X. (2009). Insecticidal activity of basil oil, trans-anethole, estragole, and linalool to adult fruit flies of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae*. *Journal of Economic Entomology*, *102*(1), 203-209.

Dejean, A., Kenne, M., & Moreau, C. S. (2007). Predatory abilities favour the success of the invasive ant *Pheidole megacephala* in an introduced area. *Journal of Applied Entomology*, *131*(9-10), 625-629.

Fournier, D., De Biseau, J. C., & Aron, S. (2009). Genetics, behaviour and chemical recognition of the invading ant *Pheidole megacephala*. *Molecular Ecology*, *18*(2), 186-199.

George, J., Bais, H.P., & Ravishankar, G. A. (2000). Biotechnological production of plant-based insecticides. *Critical Reviews in Biotechnology*, *20*(1), 49-77.

Greaves, J. H. (1989). *Rodent pests and their control in the Near East*. Rome, Italy: Food and Agriculture Organization of the Unite Nations.

Handa, S. S. (2008). An overview of extraction techniques for medicinal and aromatic plants. In S. S. Handa, S. P. S. Khanuja, G. Longo, & D. D. Rakesh (Eds.), *Extraction technologies for medicinal and aromatic plants* (pp. 21-52). Trieste, Italy: International Centre for Science and High Technology.

Hanna, R., Kuate, A. F., Nanga, S. N., Tindo, M., & Nagel, P. (2015). Boric acid for suppression of the ant *Anoplolepis tenella* and effects on an associated scale insect pest *Stictococcus vayssierei* in cassava fields in the Congo Basin. *Crop Protection*, *74*, 131-137.

Hebling, M. J. A., Bueno, O. C., Maroti, P. S., Pagnocca, F. C., & Da Silva, O. A. (2000). Effects of leaves of *Ipomoea batatas* (Convolvulaceae) on nest development and on respiratory metabolism of leaf-cutting ants *Atta sexdens* L. (Hym., Formicidae). *Journal of Applied Entomology*, *124*(5-6), 249-252.

Hinton, P. R., McMurray, I. , & Brownlow, C. (2014). *SPSS explained.* London, United Kingdom: Routledge.

Hoffmann, B. D., & O'Connor, S. (2004). Eradication of two exotic ants from Kakadu National Park. *Ecological Management and Restoration*, *5*(2), 98-105.

Hughes, W. O. H., 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. Series B: Biological Sciences*, *269*(1502), 1811-1819.

Khater, H. F. (2012). Prospects of botanical biopesticides in insect pest management. *Pharmacologia*, *3*(12), 641-656.

Klotz, J. H., Hansen, L., Pospischil, R., & Rust, M. (2008). *Urban ants of North America and Europe: identification, biology, and management.* Ithaca, NY: Comstock Publishing Associates Cornell University Press.

Kumar, S., Wahab, N., & Warikoo, R. (2011). Bioefficacy of *Mentha piperita* essential oil against dengue fever mosquito *Aedes aegypti. Asian Pacific Journal of Tropical Biomedicine*, *1*(2), 85-88.

Lee, C. Y. (2002). Tropical household ants: Pest status, species diversity, foraging behavior, and baiting studies. *Proceedings of the 4th Interntional Conference on Urban Pests* (pp. 3-18). Blacksburg, USA: Pocahontas Press.

Lee, C. Y., & Tan, E. K. (2004). *Guide to urban pest ants in Singapore*. Singapore: Singapore Pest Management Association.

Loh, F. S., Awang, R. M., Omar, D., & Rahmani, M. (2011). Insecticidal properties of *Citrus hystrix* DC leaves essential oil against *Spodoptera litura* fabricius. *Journal of Medicinal Plants Research*, *5*(16), 3739-3744.

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.

Nor, O. M. (1999). Volatile aroma compounds in *Citrus hystrix* oil. *Journal of Tropical Arigculture and Food Science*, *27*(2), 225-229.

Pavela, R., Sajfrtová, M., Sovová, H., Bárnet, M., & Karban, J. (2010). The insecticidal activity of *Tanacetum parthenium* (L.) Schultz Bip. extracts obtained by supercritical fluid extraction and hydrodistillation. *Industrial Crops and Products*, *31*(3), 449-454.

Tawatsin, A., Wratten, S. D., Scott, R. R., Thavara, U., & Techadamrongsin, Y. (2001). Repellency of volatile oils from plants against three mosquito vectors. *Journal of Vector Ecology*, *26*, 76-82.

Walker, T. N., & Hughes, W. O. H. (2009). Adaptive social immunity in leaf-cutting ants. *Biology Letters*, *5*(4), 446-448.

Williams, D. F., Collins, H. L., & Oi, D. H. (2001). The red imported fire ant (Hymenoptera: Formicidae): An historical perspective of treatment programs and the development of chemical baits for control. *American Entomologist*, *47*(3), 146-159.