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THE REPELLENT AND LETHAL EFFECTS OF BLACK PEPPER (PIPER NIGRUM), CHILLI PEPPER (CAPSICUM ANNUUM) AND CINNAMON (CINNAMOMUM ZEYLANICUM) EXTRACTS TOWARDS THE ODOROUS HOUSE ANT (TAPINOMA SESSILE)

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ABSTRACT

Tapinoma sessile is the second most dominant ant pest in Malaysia. Synthetic pest repellent effectively protects against pest. However, there is a problem because the use of synthetic repellent has led to accumulation of residue and it is toxic to human. Plant based insect repellents are believed to be comparable or better alternatives to synthetic repellent. In this study, chilli (Capsicum annuum), black pepper (Piper nigrum) and cinnamon (Cinnamomum zeylanicum) extracts were tested for repellence and lethal activity on Tapinoma sessile. In plant extraction process, a powdered plant sample was soaked in ethanol and the solvent was removed using rotary evaporator to obtain plant extracts. Filter paper tests were carried out to evaluate the repellence and lethal activity of plant extracts at 10% (v/v), 25% (v/v) and 50% (v/v) concentration towards T. sessile for three hours of exposure respectively. Statistical analysis of one way ANOVA, Tukey test and Probit analysis were performed. The treatment by C. annuum at 25% (v/v) concentration showed the most efficient repellent activity as its effectiveness is comparable to repellence activity of all three plant extracts at 50% concentration which is >90% repellence. For lethal activity, the findings revealed that C. annuum has the strongest insecticidal activity among the three plants with LC99 value 32% compared to C. zeylanicum and P. nigrum (both LC99are 41%). Based on the findings, all three plants are used in this study might be considered to be effective repellents and insecticides against T. sessile. This study provides new alternatives to dangerous synthetic ant repellents and insecticides that commonly used worldwide.

Keywords: tapinoma sessile, capsicum annuum, piper nigrum, cinnamomum zeylanicum, repellent, insecticidal, mortality, probit.

INTRODUCTION

Ant species for example the Argentine ant has exploit urban, agricultural, and natural habitats all around the world, disrupting ecosystem processes and causing economic loss [18]. Tapinoma sessile known as the odorous house ant which is a native ant species commonly found in North America [3], and also one of the household ant pests in Malaysia [12]. Some characteristics of T. sessile are dark brown body and femur with lighter colouration of tibia and tarsi [13]. There are many ways to control ant infestation. However, it is still insufficient and using conventional methods to control the ants not only resulted in the contamination of the environment but also cause colonies fragmentation that increases the number of nests of the species [6]. N, N-diethyl-m-toluamide (DEET) is the most effective and most widely used as insect repellent available in the market [9]. However, DEET has detrimental effects apart from its benefits [1]. A previous study has proven that some people exposed to DEET developed symptoms such as drowsiness, headache, vomiting, rashes and seizures which indicate of DEET poisoning [4]. Therefore, to replace DEET, plant extracts based repellents are always considered as "safer" or "natural" alternatives [20]. Black pepper, chilli and cinnamon have high repellency against wheat grain pest [19], cotton pest [11] and malaria vector [5] respectively. Research efforts for natural and eco-friendly repellents are rising and based on researches, it is known that some plant-based repellents are comparable to synthetics, or even better [14]. Therefore, this study was performed to find out the effectiveness of plant extracts as repellents and insecticides to fight against T. sessile.

MATERIALS AND METHODS

Plant extraction

Chilli pepper (C. annuum), cinnamon (C. zeylanicum) and black pepper (P. nigrum) were obtained from local market located in Arau, Perlis. Chilli was dried in oven at 35 ºC overnight [21]. All samples blended into powder separately using electrical blender. 50g for each sample was added to 500ml of ethanol [23] and placed in orbital shaker for 48 hours. The ethanolic extracts were filtered using Whatman No.1 filter paper by vacuum filtration. The solvent removed using rotary evaporator at 40ºC. Extracts were stored in amber bottle. Each plant extract was diluted to 10% (v/v), 25% (v/v) and 50% (v/v) concentration with ethanol.

Repellence test

Granulated sugar in a bottle used as bait to collect ants from ant colonies [2]. To pick ants from the bottle, it was placed in a freezer for one minute to reduce its activity. Filter paper test was used to test repellence

activity of extract. Filter paper was cut into halves. One part treated with 0.5 ml diluted extract and another part is treated with 0.5 ml ethanol. After the filter paper fully dried, it was placed in a petri dish. Vaseline was applied to the inner wall of petri dish to avoid ants from escaping. Eight ants were introduced in the middle between the two parts of filter paper. The number of individual ants present on extract treated filter paper was observed at 5 minutes intervals for 180 minutes to test the effect of concentration towards repellent activity. For each plant extract, the procedure repeated with concentration of extract 0% w/v, 10% (v/v), 25% (v/v) and 50% (v/v) three times. The percentage of repellency calculated using Equation. (1):

$$\text{Repellency (\%)} = (C - E) / T \quad (1)$$

where C is the ant number on control area, E is the ant number on treated area and T is the total ant number [10].

Lethal test

A filter paper was treated with 2ml of diluted extract. After the filter paper fully dried, it was placed in a petri dish. Vaseline was applied to the inner wall of petri dish to avoid ants from escaping. Eight ants were introduced in the middle of filter paper. After 3 hours of exposure, the number of dead ants was counted to test the effect of concentration towards insecticidal activity [12]. For each plant extract, the procedure repeated with concentration of extract 0% (v/v), 10% (v/v), 25% (v/v) and 50% (v/v) three times.

Statistical analysis

The relationship between concentrations of extracts and repellence activity was determined using Pearson correlation. Comparison of repellence activity between each plant type was done using one way ANOVA. The effectiveness of each plant extract at different concentration as ant repellents were evaluated through a one-way ANOVA analysis and significant differences between the concentrations were tested by Tukey's test. Probit analysis was done to determine LC50.

RESULTS AND DISCUSSIONS

In this study, three plant types were tested for their repellence and lethal activity at different concentrations against T. sessile. Pearson correlation test was performed in order to find out correlation between concentration and repellency of C. annuum, P. nigrum and C. zeylanicum extracts. The results were tabulated in Table-1.

Table-1. Relationship between different concentrations of each plant extracts.

Plant	Pearson correlation	Sig. (2-Tailed)	N
Capsicum annuum	0.889	0.111	4
Piper nigrum	0.915	0.085	4
Cinnamomum zeylanicum	0.925	0.075	4

From the results of the analysis, all three plants showed no statistically significant correlation between concentration and repellence activity of the extracts because the P-value obtained for all plants were greater than 0.05. However, the findings are demonstrated a strong relationship between the two variables of each plant because Pearson's r value obtained for all plants were close to 1.000. Therefore, the repellence activity of each plant extract cannot be considered increasing with the increment of concentration. The repellent activity reached plateau at certain concentration as shown in Figure-1.

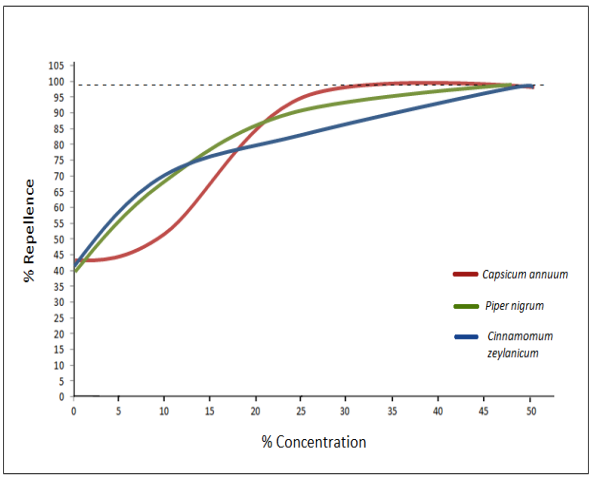

Figure-1. Relationship between concentration and repellence activity of three extracts.

Table-2 demonstrates the repellence activity of the three plants extracts. C. annuum at 50% concentration showed the highest repellence effect and successfully repelled 99.67 ± 0.577% ant samples. Both P. nigrum and C. zeylanicum at 50% concentration also demonstrated very strong repellence activity by 99.00 ± 0.000% and 98.67 ± 0.577% repellency respectively. This shows that all three plants have the strongest repellent at 50% concentration. At 25% concentration, C. annuum has 98.00 ± 1.732% repellency which was the highest compared to P. nigrum and C. zeylanicum with 90.00 ± 5.292% and 83.00 ± 3.000% respectively. However, C. zeylanicum has strong repellence by 71.33 ± 1.155% at 10% concentration but P. nigrum and C. annuum were only able to repel ants sample moderately by 67.33 ± 3.055% and 52.33 ± 4.933% respectively at the same

concentration. This shows that at lower concentration, C. zeylanicum can effectively kill more ants compared to other two plants. Through one way ANOVA analysis where P-value was above 0.05, it indicates that there was no significant difference between the repellence activities of the three plants.

Table-2. Repellence activity of Capsicum annuum, Piper nigrum and Cinnamomum zeylanicum extracts at 0%, 10%, 25% and 50% concentrations and comparison of repellence activity between plants.

Plant	Conc. % (v/v)	% Repellence (Mean ± SD)	P-value
Capsicum annuum	0	44.33 ± 2.517	1.000
	10	52.33 ± 4.933	
	25	98.00 ± 1.732	
	50	99.67 ± 0.577	
Piper nigrum	0	39.33 ± 6.658	
	10	67.33 ± 3.055	
	25	90.00 ± 5.292	
	50	99.00 ± 0.000	
Cinnamomum zeylanicum	0	40.67 ± 9.504	
	10	71.33 ± 1.155	
	25	83.00 ± 3.000	
	50	98.67 ± 0.577	

*The mean difference is significant at the 0.05 level

Tukey test was performed and Table-3 demonstrates the multiple comparisons of different concentrations of each plant type. From the multiple comparisons, for C.annuum and P. nigrum, it was found that there was no significant difference in repellence activity between 25% and 50% concentration (P-value 0.984 and 0.146 respectively). This result indicates that both plants at 25% and 50% concentration have similar effectiveness in repelling T. sessile. Therefore, the most efficient concentration for both plants wasat 25%. For C. zeylanicum, there was no significant difference in repellency at 10% and 25% concentration which indicates similarity in their repellence activity.

Table-3. Multiple comparisons of repellent activity between different extract concentrations within each plant type.

Plant	Concentrations, % (v/v)		P-value
Capsicum annuum	0	10	0.040*
		25	0.000*
		50	0.000*
	10	25	0.000*
		50	0.000*
	25	50	0.984
Piper nigrum	0	10	0.000*
		25	0.000*
		50	0.000*
	10	25	0.001*
		50	0.000*
	25	50	0.146
Cinnamomum zeylanicum	0	10	0.000*
		25	0.000*
		50	0.000*
	10	25	0.083
		50	0.001*
	25	50	0.021*

*The mean difference is significant at the 0.05 level

Probit analysis was done in order to determine lethal concentration, LC50 and LC99 of each plant extract and the results tabulated in Table-4. Both P. nigrum and C. zeylanicum have LC50 of 15%. C. annuum has higher LC50 which was 18%. However, LC99 value of C. annuum (32%) was lower than LC99 value of both P. nigrum and C. zeylanicum (41%). This shows that C. annuum was more effective as insecticide compared to P. nigrum and C. zeylanicum because the plant required less 9% concentration of extract in order to kill 99% of total number of ant sample.

Table-4. LC50 and LC99 of three plants extracts.

Plant	LC_{50} (%)	LC_{99} (%)
Capsicum annuum	18	32
Piper nigrum	15	41
Cinnamomum zeylanicum	15	41

The findings of this study are supported by previous studies. C. annuum showed effective repellency against several species of stored products pests such as Sitophilus zeamais and Tribolium castaneum, also act as insecticidal against Sitotroga cerealella and also as biopesticides against larvae of Alfalfa weevil (Hypera postica) [15]. Capsaicin is the most plentiful out of all the phenolic compounds which made up 37.22% of total capsinoid content in C. annuum [22]. Piperamides extracted from different species of piper including Piper nigrum are the active compounds contribute to insecticidal activity [17]. In a study done by [16] reported that ingestion of pipercide from P. nigrum extract has effectively killed common forest insect pests such as

Lymantria dispar and Malacosoma disstria which are found in north-eastern North America. According to [7] reported that limonene content in plant essential oils is responsible for repellent activity. Other than mosquito, C. zeylanicum has also been proven to have positive repellency against the bean weevil, Acanthoscelides obtectus, in small storage units [8].

CONCLUSIONS

It can be concluded that all three plants have similarity in effectiveness to repel T. sessile. However, there was significant difference in repellent activity at certain concentrations of each plant. The repellent activity of each extract reached plateau at certain higher concentrations. Besides, C. annuum at 25% concentration can be considered as the most efficient repellent because at lower concentration, its repellence activity was comparable to all three plants extracts at 50% concentration. Based on LC99, it can be concluded that the insecticidal activity of both C. annuum was better than both C. zeylanicum and P. nigrum.

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