

REPELLENT ACTIVITY OF ESSENTIAL OILS AGAINST COCKROACHES (DICTYOPTERA: BLATTIDAE, BLATTELLIDAE, AND BLABERIDAE) IN THAILAND

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Abstract. Seven commercial essential oils extracted from the plant species *Boesenbergia rotunda* (L.) Mansf., *Citrus hystrix* DC., *Curcuma longa* L., *Litsea cubeba* (Lour.) Pers., *Piper nigrum* L., *Psidium guajava* L. and *Zingiber officinale* Roscoe, and naphthalene as a control, were evaluated for repellent activity against the three cockroach species *Periplaneta americana* (L.), *Blattella germanica* (L.) and *Neostylopyga rhombifolia* (Stoll) under laboratory conditions. The essential oil derived from *Citrus hystrix* showed the best repellency over other candidate essential oils and naphthalene. The essential oil of *Citrus hystrix* exhibited complete repellency (100%) against *P. americana* and *B. germanica*, and also showed the highest repellency (among the essential oils tested) of about 87.5% against *N. rhombifolia* under laboratory conditions. In the field, *Citrus hystrix* essential oil formulated as a 20% active ingredient in ethanol and some additives provided satisfactory repellency of up to 86% reduction in cockroaches, mostly *P. americana* and *N. rhombifolia* with a residual effect lasting a week after treatment. *Citrus hystrix* essential oil has good potential for being used as a cockroach repellent. Further improvements in efficacy and residual activity may be realized with appropriate formulations.

INTRODUCTION

Cockroaches have the potential to mechanically carry and transmit many pathogens, such as bacteria, viruses, fungi, protozoa and helminthes (Cochran, 1982). They also serve as potential carriers of the causes of bacterial diarrhea and nosocomial infections in hospi-

tals (Agbodaze and Owusu, 1989; Fotedar *et al*, 1991). There is ample evidence that substances produced by cockroaches are involved in producing allergic symptoms (Kongpanichkul *et al*, 1997; Pumhirun *et al*, 1997). At least 12 species of cockroaches have been reported in Thailand to date and the American cockroach is the most common cockroach found in dwellings in various provinces of Thailand (Tawatsin *et al*, 2001). Sriwichai *et al* (2002) confirmed the abundance of the American cockroach in all types of buildings in Bangkok, especially private residences, and found that German cockroaches were significantly predominant in grocery establishments.

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Several chemicals were studied for repellent effects against cockroaches, such as N, N-diethylphenylacetamide (Prakash *et al*, 1990), methyl neodacanamide, propyl neodecanamide, methyl neotridecanamide, alkyl and aryl neoalkanamides (Steltenkamp *et al*, 1992), citral and eugenol (Vartak *et al*, 1994). Research regarding cockroach repellents, especially those derived from plant extracts, is quite limited at this time. Recently, the essential oil of catnip (*Nepeta cataria* L.) was reported as having repellency against adult male German cockroaches (Peterson *et al*, 2002). Up to the present time, no studies have reported evaluation of repellents against cockroaches in Thailand. The present study was initiated to study the repellent activity of seven essential oils, extracted from local plants of Thailand, against three cockroach species under laboratory conditions. The most promising essential oil was then further evaluated for repellency against cockroaches in the field.

MATERIALS AND METHODS

Essential oils for laboratory and field evaluation

Seven commercial essential oils (100%) were evaluated for repellent activity against three cockroach species (the American cockroach, the German cockroach and harlequin cockroach) under laboratory conditions. These essential oils were derived from lesser galanga (*Boesenbergia rotunda* (L.) Mansf.) rhizomes, kaffir lime (*Citrus hystrix* DC.) leaves, turmeric (*Curcuma longa* L.) rhizomes, Litsea (*Litsea cubeba* (Lour.) Pers.) fruits, black pepper (*Piper nigrum* L.) fruits, guava (*Psidium guajava* L.) leaves and ginger (*Zingiber officinale* Roscoe) rhizomes. These oils were selected for this study because the plants are commonly available in Thailand and the oils are available commercially. These oils were purchased from Thai-China Flavours and Fragrances Industry Co, Ltd, Nonthaburi Province, Thailand. Naphthalene was used as a

control since it is commonly used as cockroach repellent.

The essential oil of kaffir lime leaf was selected for further evaluation against cockroaches in the field because of its high efficacy under laboratory conditions. This oil was prepared at various concentrations in ethanol [5, 10, 20, 50 and 100% (undiluted)] and tested against two cockroach species (the American cockroach and the German cockroach) under laboratory conditions. For field testing, the essential oil was then formulated as 20% (w/w) liquid repellent in ethanol with some additives.

Test cockroaches

Three cockroach species used in laboratory repellent tests were laboratory-reared *Periplaneta americana* (L.) (the American cockroach), *Blattella germanica* (L.) (the German cockroach) and *Neostylopyga rhombifolia* (Stoll) (Harlequin cockroach). These cockroaches have been reared according to the standard protocols of the Biology and Ecology Section, National Institute of Health, Thailand, and maintained in the insectary of the institute. The colonies were maintained in the insectary under ambient temperature (24-30°C) and humidity (70-90% RH). Adult *P. americana* (aged 3-5 months), *B. germanica* (aged 6-8 weeks) and *N. rhombifolia* (aged 3-5 months) were employed for repellent testing under laboratory conditions. Both males and females of each cockroach species were used in the laboratory tests.

Laboratory tests

A stainless steel square-box (50x50x15 cm, with the top open) was employed in the repellent tests. All four walls of the box were smeared with Vaseline to prevent escape of cockroaches. A piece of filter paper (Whatman No.1, 50x50 cm) was marked by a pen to divide it into 2 equal parts (treated and control areas) and then placed at the bottom of the

box. The test repellent (1.25 ml) was applied (equal to dosage of 10 ml/m²) by placing drops from a pipette on the treated area to cover the treated portion of the paper, whereas the control area was untreated. Naphthalene, the control standard, is solid. Two pieces of naphthalene (1 g each) were placed together as a treatment on the treated side. Each set of containers of food and drink for the cockroaches was placed at both sides of the box (treated and control areas). Twenty adult cockroaches (10 males and 10 females) were anesthetized with CO₂ and released into the box at the central point. The box was then placed in a Peet Grady chamber (180x180x180 cm) surrounded by cloth curtains to keep a dark environment and to prevent disturbances from surroundings. The cockroaches located in the treated and control areas were carefully observed and counted at 48 hours after treatment. Repellency against the cockroaches was calculated with the following equation:

$$\text{Repellency (\%)} = 100 - \frac{[T \times 100]}{N}$$

where T stands for the number of cockroaches located in the treated area and N stands for the total number of cockroaches used. The average repellency was calculated from the values obtained in six replicates.

Field evaluations – village-scale trials

The essential oil of kaffir lime (*Citrus hystrix*) leaves was selected for further evaluation in the field because it showed the highest repellent activity against the three cockroach species tested under laboratory conditions. Field evaluation of the repellent formulated from the essential oil of kaffir lime leaves was carried out in Pitsanulok Province, Thailand. Three villages in rural areas were selected where the experiment was carried out. Kok Makham Yai Village of Wang Thong District (51 houses) and Wang Itok Village of Bang Rakam

District (36 houses) were designated as the treatment sites, whereas Bang Saphan Village of Wang Thong District (50 houses) was designated as the control site. All sites were surveyed for cockroach species and densities using sticky traps (HOY HOY, produced by Earth Chemicals, Japan) before and after treatment. This sticky trap has been shown to be an effective tool for cockroach surveys in the field (Tawatsin *et al*, 2001). The sticky trap is a simple device that can be folded into a trapezoid paper-house (10x15x3 cm), having four entrances for cockroaches. The sticky area for catching cockroaches inside the trap is about 9.5x15 cm. Cockroaches are lured into the trap by built-in attractants located in the middle of the sticky area. At least 36 houses in each experimental site were randomly sampled for cockroaches by placing two sticky traps in the kitchen of each house and left there for one night. The cockroaches caught in each trap were identified by species following the handbook of domiciliary cockroach species in Thailand (Asahina, 1983) and other relevant references (Cornwell, 1968; Bell, 1981; Cochran, 1982, 1999) and then counted. Three days after the preliminary survey, the test repellent was applied at a dosage of 10 ml/m² by a hand-trigger window-sprayer on the floor in the kitchen area in each house of the two treated sites, whereas the houses in the control site were untreated. The treatment was carried out only once in each house of the two treated sites. To assess the degree of cockroach infestation, the sticky traps were again placed in the kitchens of each house at the three experimental sites and left there for one night. Then, all the traps were collected and the cockroaches caught in each trap were identified by species and counted. Assessment of the cockroach densities and species in each house at the three experimental sites was carried out on three additional occasions at 6-, 9- and 12-days post-treatment.

Another field evaluation of the repellent formulated from the essential oil of kaffir lime leaves was conducted in Bang Khaen District, Bangkok, Thailand. Two urban communities (one treated and one control site) were selected for this evaluation. Bang Khaen-1 (the treated site) included 44 houses, whereas Bang Khaen-2 (the control site) consisted of 37 houses. The evaluation carried out in the field in Bangkok was similar to that in Pitsanulok as described above. However, the assessments were carried out once a week for four weeks post-treatment during the course of this study. After each weekly assessment, the repellent was reapplied at the same dosage in the previously treated areas in each house of the treated sites.

The average number of collected cockroaches per house (mean no.) and standard error of the mean (SE) were calculated for each study site in each assessment in the field. The percentage reduction in cockroach number following treatment at each treated site was calculated by Mulla's formula (Mulla *et al*, 1971):

$$\text{Reduction (\%)} = 100 - [(C_1/T_1) \times (T_2/C_2)] \times 100$$

where: C_1 = average number of cockroaches per house at the control site (pre-treatment),

T_1 = average number of cockroaches per house at the treated site (pre-treatment),

C_2 = average number of cockroaches per house at the control site (post-treatment),

T_2 = average number of cockroaches per house at the treated site (post-treatment).

These values, mean SE and percentage reduction (%), are presented in the figures.

Data analysis

Comparison of repellency among test repellents was carried out employing the one-way analysis of variance (ANOVA) with Duncan's multiple range test. All differences were considered significant at $p \leq 0.05$.

RESULTS

Laboratory repellency

The essential oil of *Citrus hystrix* provided complete repellency (100%) against *P. americana* cockroaches, and a high degree of repellency was also obtained from the essential oils of *Psidium guajava* (95%) and *Boesenbergia rotunda* (90%) (Table 1). Moderate levels of repellency were derived from the essential oil of *Litsea cubeba* (88.3%) and *Zingiber officinale* (85%). The essential oil of *Curcuma longa* and *Piper nigrum* exhibited repellency equal to that of naphthalene (80%).

The essential oils of *Citrus hystrix* and *Psidium guajava* showed excellent repellency (100%) against *B. germanica* cockroaches in the laboratory (Table 1). However, high degrees of repellencies against cockroaches were also obtained from the essential oils of *Boesenbergia rotunda* (95%), *Curcuma longa* (95%), *Piper nigrum* (95%), *Zingiber officinale* (95%) and *Litsea cubeba* (90%). It is interesting to note that all the essential oils in this experiment provided better repellencies than did the standard repellent naphthalene (85%).

Regarding the repellent tests against *N. rhombifolia* cockroaches, the essential oil of *Citrus hystrix* exhibited the highest repellency (87.5%) of the tested repellents, whereas the essential oil of *Curcuma longa* provided the lowest repellency of about 50.8% (Table 1). Moderate repellencies against *N. rhombifolia* were obtained from the essential oils of *Zingiber officinale* (70%), followed by those of *Litsea cubeba* (67.5%), naphthalene (65%), *Boesenbergia rotunda* (60.8%), *Piper nigrum* (58.3%) and *Psidium guajava* (56.7%). There were no significant differences among repellencies obtained from this group.

The repellency of essential oil of *Citrus hystrix* at various concentrations against *P. americana* and *B. germanica* is shown in Fig 1. The 50% concentration and undiluted essential oil provided excellent repellency from

Table 1
Repellency of essential oils and naphthalene against *P. americana*, *B. germanica* and *N. rhombifolia* cockroaches in the laboratory.

Plant essential oils/chemical	Mean repellency* (%) ± SE		
	<i>P. americana</i>	<i>B. germanica</i>	<i>N. rhombifolia</i>
<i>Boesenbergia rotunda</i>	90 ± 2.6 c	95 ± 1.8 b	60.8 ± 7.7 bc
<i>Citrus hystrix</i>	100 ± 0.0 a	100 ± 0.0 a	87.5 ± 5.3 a
<i>Curcuma longa</i>	80 ± 1.8 e	95 ± 1.3 b	50.8 ± 8.0 c
<i>Litsea cubeba</i>	88.3 ± 3.3 cd	90 ± 2.9 c	67.5 ± 8.6 b
<i>Piper nigrum</i>	80 ± 1.3 e	95 ± 1.8 b	58.3 ± 7.0 bc
<i>Psidium guajava</i>	95 ± 1.8 b	100 ± 0.0 a	56.7 ± 9.4 bc
<i>Zingiber officinale</i>	85 ± 2.2 d	95 ± 1.3 b	70.0 ± 8.1 b
Naphthalene	80 ± 1.8 e	85 ± 2.9 c	65.0 ± 9.2 bc

*Repellency against the same species (in the same column) followed by the same letter is not significantly different from each other ($p \geq 0.05$, by one-way ANOVA and Duncan's multiple range test).

95 to 100% against *P. americana* cockroaches. The essential oil at 20% concentration exhibited a moderate level of repellency at an average of about 83.5%. The essential oil diluted to 10% and 5% showed lower repellency against *P. americana*, about 60% and 58.5%, respectively. Regarding repellency against *B. germanica*, the 50% concentration and the undiluted essential oil of *Citrus hystrix* also provided excellent repellency (93-100%), whereas the essential oil at 20% concentration showed an average repellency of about 85%. The results against *B. germanica* were similar to those against *P. americana*, the essential oil diluted to 10% and 5% exhibited repellency of 61.5% and 50% against the *B. germanica* cockroaches, respectively.

Field repellent test against cockroaches in Pitsanulok Province

Kok Makham Yai Village (the 1st treated site). A total of 51 houses in the first treated site (Kok Makham Yai Village) were surveyed and a total of 194 cockroaches were found in 31 houses (60.8% positive) during the preliminary inspection before treatment. The average number of cockroaches collected at Kok

Makham Yai Village prior to treatment was 3.8 cockroaches/house (Fig 2). Three days after treatment with the test repellent (20% *Citrus hystrix*), 138 cockroaches were caught from 21 houses (41.2% positive). The average number of captured cockroaches dropped to 2.7 cockroaches/house with a reduction of about 21.3% using the formula of Mulla *et al* (1971), taking both the treated and control populations (Fig 2). Subsequently, 19 houses (37.3%) were positive for 119 cockroaches during the inspection at 6 days after treatment. An average of 2.3 cockroaches/house was obtained in this assessment with 17.3 % reduction (Fig 2).

The number of houses infested with cockroaches declined to 17 houses (33.3% positive) at 9 days post-treatment with only 59 cockroaches found in this survey. As shown in Fig 2, the mean number of captured cockroaches dropped to 1.2 cockroaches/house with the reduction reaching a peak of 66.8%. Finally, 20 houses (39.2%) were positive for a total of 76 cockroaches in the last period of survey (12 days post-treatment). The reduction remained at 52.4 % with an average of 1.5 cockroaches/house (Fig 2).

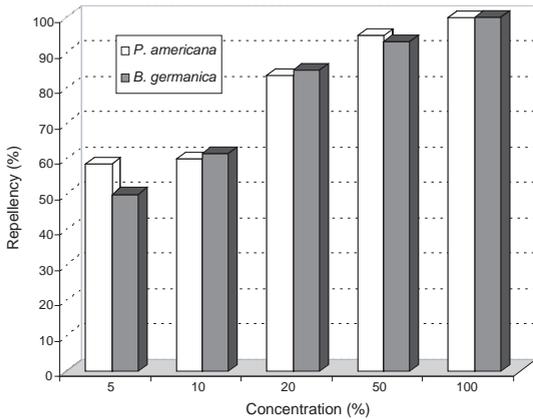


Fig 1—Comparison of repellency of essential oil of kaffir lime (*Citrus hystrix*) leaves at various concentrations in ethanol against *P. americana* and *B. germanica* cockroaches under laboratory conditions.

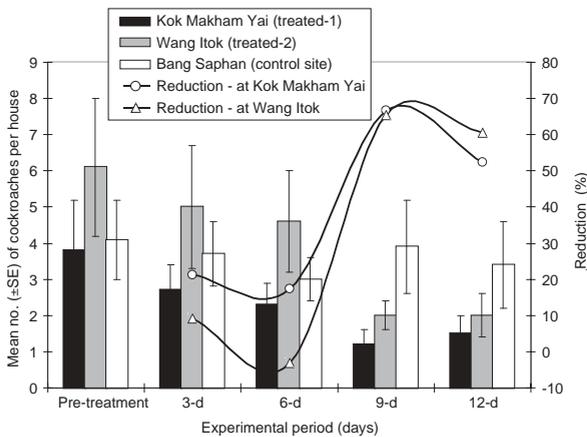


Fig 2—Field evaluation of formulated repellent containing 20% essential oil of kaffir lime (*Citrus hystrix*) against cockroaches conducted in three villages of Pitsanulok Province, Thailand.

There were seven species of cockroaches found at Kok Makham Yai Village during the five surveys taken during the course of this study. The predominant species was *P. americana* (42.8-84.9%), followed by *N. rhombifolia* (13.5-34.2%), *P. brunnea* Burmeister (0.7-30%), *Pycnoscellus surinamensis* (Lin-

neaus) (1.7-18.9%), *Supella longipalpa* (F.) (0.8-10.3%), *B. germanica* (0.7-7.9%) and *P. australasiae* (Fab.) (0.5-2.6%).

Wang Itok Village (the second treated site). The preliminary survey conducted at Wang Itok Village (the second treated site) revealed that 26 (72.2%) of 36 houses were infested with a recovery of 221 cockroaches. The average number of cockroaches captured at Wang Itok Village before treatment was 6.1 cockroaches/house (Fig 2). A total of 20 houses (55.6%) were positive for cockroaches 3 days after treatment with the test repellent (20% *Citrus hystrix*) and 179 cockroaches were captured. An average of 5 cockroaches/house was obtained in this survey with a reduction rate of 9.2% according to Mulla's formula compared to pretreatment at the control site (Fig 2). The numbers of houses infested with cockroaches decreased to 18 houses (50% positive) during the survey carried out 6 days post-treatment with a total of 166 cockroaches. The average number of cockroaches captured in this assessment was 4.6 cockroaches/house, a reduction of 3.1% (Fig 2).

In the survey conducted 9 days after treatment, 16 houses (44.4%) were positive for cockroaches and only 72 cockroaches were collected. An average of 2 cockroaches/house were found in this survey. The reduction reached a peak of about 65.5% (Fig 2). Twelve days after treatment, the number of houses infested with cockroaches decreased to 11 houses (30.6% positive) with 73 cockroaches captured. As seen in Fig 2, the average number of collected cockroaches was about the same as the previous survey (~2 cockroaches/house), however, the reduction declined to 60.5%.

Eight species were identified from cockroaches caught from Wang Itok Village from five surveys in this study. *P. americana* (47.5-87%) was the predominant species found in all surveys, followed by *N. rhombifolia* (7.3-

31.7%), *S. longipalpa* (2.2-18.1%), *B. lituricollis* (Walker), *B. germanica*, *P. brunnea*, *Nauphoeta cinerea* (Olivier) and *Py. surinamensis* were found less than 2%.

Bang Saphan Village (control site). Cockroach surveys were also carried out in Bang Saphan Village (control site) during the same period as the trial in Kok Makham Yai Village and Wang Itok Village. On a preliminary survey, 32 out of 50 houses (64%) were positive for cockroaches. A total of 206 cockroaches were caught in this survey with an average of 4.1 cockroaches/house (Fig 2). Three days after the preliminary survey, the number of houses infested with cockroaches declined to 21 houses (42%) and 187 cockroaches were caught (average 3.7 cockroaches/house). Later, 25 houses (50%) were positive for cockroaches in the survey conducted 6 days after the preliminary survey and 150 cockroaches were captured during this inspection. An average number of 3 cockroaches/house was obtained in this inspection.

A total of 197 cockroaches were found in 24 houses (48% positive) in the control site in the fourth survey carried out nine days after the preliminary survey, with an average of 3.9 cockroaches/house. Finally, 24 houses (48%) were still positive with a total of 168 cockroaches at the last inspection (12 days after the preliminary survey). The average number of captured cockroaches remained at 3.4 cockroaches/house (Fig 2).

Seven species of cockroaches were collected from Bang Saphan Village (control) during five surveys in this study. These included *P. americana* (34.5-70%), *N. rhombifolia* (24-60.7%), *S. longipalpa* (2.4-16.3%), *B. germanica* (0.5-4.6%), *P. brunnea* (1-2%), *P. australasiae* (0.6-1%) and *Py. surinamensis* (0.5-1%).

Field repellent test against cockroaches in Bangkok

Bang Khaen-1 Community (the treated site).

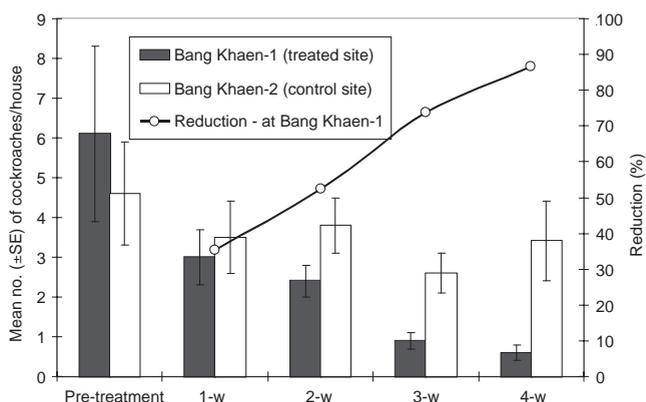


Fig 3—Field evaluation of formulated repellent containing 20% essential oil of kaffir lime (*Citrus hystrix*) leaves applied weekly against cockroaches in two urban communities of Bang Khaen District, Bangkok, Thailand.

A total of 28 (63.6%) out of 44 houses were positive for cockroaches in the pre-treatment survey carried out in the treated site (Bang Khaen-1 Community) and 268 cockroaches were collected in this survey with an average number of 6.1 cockroaches/house (Fig 3). One week after treatment with the test repellent (20% *Citrus hystrix*), although the number of houses infested with cockroaches still remained at 28 (63.6%) similar to that of the pre-treatment survey, the number of collected cockroaches had declined to 131. As a result, the average number dropped to 3 cockroaches/house with a 35.4% reduction. The number of infested houses decreased slightly to 25 (56.8%) in the survey conducted two weeks post-treatment and 107 cockroaches were captured. In this inspection, an average number of 2.4 cockroaches/house was obtained, whereas the reduction rate increased to 52.4% (Fig 3).

In the survey carried out three weeks after treatment, the number of houses positive for cockroaches declined to 13 (29.5%) and 40 cockroaches were collected. As shown in Fig 3, the average number of 0.9 cockroaches/

house was achieved with a reduction of about 73.9%. Finally, the number of houses infested with cockroaches remained at 10 (22.7%) in the final inspection conducted four weeks post-treatment and 27 cockroaches were captured in this survey. An average number of 0.6 cockroaches/house was found in this assessment with a high reduction rate of 86.7% (Fig 3).

There were only five species of cockroaches collected from the five surveys carried out at Bang Khaen-1 Community. These were *P. americana* (28-80%), *P. brunnea* (12.5-47.8%), *N. rhombifolia* (0.7-23.4%), *B. germanica* (3-15.3%) and *S. longipalpa* (0-4.7%).

Bang Khaen-2 Community (control site). The cockroach surveys were also carried out in Bang Khaen-2 Community (the control site) during the same period as the study in Bang Khaen-1 Community for comparison. The results of pre-treatment survey showed that 26 (70.3%) out of 37 houses were infested with cockroaches and 169 cockroaches were caught. An average of 4.6 cockroaches/house was obtained from this assessment (Fig 3). One week after the preliminary survey, 20 houses (54.1%) were positive with a total of 129 cockroaches (average 3.5 cockroaches / house). Subsequently, it was found that 27 houses (73%) in the control site were infested with 142 cockroaches during the inspection at two weeks after the preliminary survey with an average of 3.8 cockroaches/house. In the third week post-treatment, the inspection revealed that 15 houses (40.5%) were positive for a total of 95 cockroaches and the average captured cockroaches declined to 2.6 cockroaches/house. Finally, 21 houses (56.8%) in the control site were found positive for cockroaches with 125 cockroaches collected in the last inspection. The average number of cockroaches at this assessment increased to 3.4 cockroaches/house.

Six species were identified from cockroaches captured in Bang Khaen-2 Community during the course of this study. These were *N. rhombifolia* (26.1-77.9%), *P. americana* (13.7-69%), *S. longipalpa* (5.8-13.9%), *P. brunnea* (4.9-8.4%), *B. germanica* (4.7%) and *N. cinerea* (0-3.2%).

DISCUSSION

The test method used in this study was developed by the Biology and Ecology Section, the National Institute of Health, Thailand, since no standard method for evaluation of cockroach repellent had been established elsewhere. It was selected because of its reliability among several attempts that had been made previously. The laboratory repellency results indicated differences in susceptibility to volatile chemicals derived from essential oils among the three species of test cockroaches. *B. germanica* was the most sensitive species in this study, followed by *P. americana*, *N. rhombifolia*. All the essential oils in this study provided equal or better repellencies against *P. americana* and *B. germanica* than naphthalene (80%). Naphthalene is the most common chemical used as a cockroach repellent. However, naphthalene is hazardous to humans. Humans exposed for long periods to naphthalene by inhalation, ingestion or dermal contact may develop hemolytic anemia, damage to the liver, or neurological damage in infants (ATSDR, 1995).

The regulation for cockroach repellent products in Thailand states there must be a minimal repellency of 80% against *P. americana*. The seven essential oils tested in this study therefore qualified against both *P. americana* and *B. germanica*. However, *Citrus hystrix* was the most effective repellent tested providing complete repellency (100%) against both *P. americana* and *B. germanica*, and the highest repellency (among all the essential oils tested) of about 87.5% against *N.*

rhombofolia. It was selected for further evaluation in the field at a dosage of 20% *Citrus hystrix*.

In the field, the cockroach repellent formulated from *Citrus hystrix* essential oil (20%) showed satisfactory repellency in the treated areas in both Pitsanulok Province and Bangkok. In Pitsanulok, the repellent activity reached its peak at nine days post-treatment at both treated sites (66.8 and 65.5% reduction). Therefore, we assessed repellent activity with weekly applications in the field evaluation carried out in Bangkok. We found that weekly surveys and re-applications of repellent were practical and effective. Although the repellent could not repel cockroaches completely, it could substantially reduce the numbers of cockroaches as well as the numbers of houses infested with cockroaches in the treated sites compared to the control sites. The infestation rate and number of cockroaches captured in the treated site in Bangkok declined substantially, especially two weeks post-treatment. This could be due to the accumulated residual activity of the repellent that was applied weekly. The reduction of cockroaches and infestation rates in the treated sites may have been partially affected by trap catching; however, this factor is minor as seen in the results of the control site.

In this study, we used Mulla's formula to assess the degree of reduction in cockroach number for each treated site following the treatment with the assumption that the treated and control sites were uniform in regard to factors contributing to changes in cockroach populations (Mulla *et al*, 1971). In practice, this formula was powerful for assessing the level of reduction of cockroaches in this study as it compares the number at the treated site and the control site both pre- and post-treatment. According to this formula, no reduction occurs in the cockroach numbers because of treatment if the factor $[(C_1/T_1) \times (T_2/C_2)]$ is

greater than 1. This phenomenon appeared once in the treated site at Wang Itok Village (6 days post-treatment). In fact, it occurred because the average number of cockroaches post-treatment at the treated site (T_2) was greater than that of post-treatment in the control site (C_2).

It is interesting to note that people at the treated sites mostly accepted this repellent and some residents acknowledged that mosquito-biting activity was reduced at night in the treated areas along with the cockroach reduction. These results indicate the potential for the use of the *Citrus hystrix* essential oil as a cockroach repellent in the future. This is the first study of repellents derived from plant extracts against cockroaches in Thailand. More research is needed to develop more effective formulations. These may include long-lasting formulations using micro-encapsulation techniques and combinations with other essential oils for synergistic effects.

Research regarding repellents against cockroaches is limited at this time, especially in agents derived from plant extracts. Recently, Peterson *et al* (2002) investigated the repellent activity of catnip essential oil (*Nepeta cataria*), two purified isomers of nepetalactone and deet (N,N-diethyl-methylbenzamide) against male German cockroaches (*B. germanica*) in a choice-test arena and found that E,Z-nepetalactone was the most active of the compounds tested, being significantly more active than equivalent doses of the essential oil, Z,E-nepetalactone, or deet. Other studies have evaluated chemicals for repellent effects against cockroaches. N,N-diethylphenylacetamide (DEPA), at a dosage of 0.5 mg/cm² showed residual repellency against *P. americana*, *B. germanica* and *S. longipalpa* for 4, 3 and 2 weeks, respectively (Prakash *et al*, 1990). Steltenkamp *et al* (1992) demonstrated that alkyl and aryl neoalkanamides with a total carbon number between 11

and 14 exhibited highly repellent effects against male *B. germanica*. In addition, methyl neodecanamide, propyl neodecanamide and methyl neotridecanamide were also found highly repellent against females and nymphs of *B. germanica*, and male *P. americana* (Steltenkamp *et al*, 1992). It is interesting to note that these chemicals showed relatively specific repellency against certain species, sexes and developmental stages of cockroaches. Vartak *et al* (1994) showed that citral and eugenol were effective as repellents against *P. americana* under laboratory conditions when used at the dosages of 25-100 mg per 4 x 4 cm filter paper. However, none of these chemicals is currently marketed as a commercial repellent product against cockroaches.

In conclusion, the essential oil derived from *Citrus hystrix* exhibited complete repellency (100%) against *P. americana* and *B. germanica*, and also showed the highest repellency (among all essential oils tested) of about 87.5%, against *N. rhombifolia* under laboratory conditions. In addition, the repellent containing 20% *Citrus hystrix* essential oil formulated in ethanol and some additives also showed satisfactory repellency yielding up to 86% reduction in cockroaches, mostly *P. americana* and *N. rhombifolia*, in field tests with residual effects for a week after treatment. The present study reveals the potential for *Citrus hystrix* essential oil to be used as a cockroach repellent. Further improvements in efficacy and longevity are expected with appropriate formulations.

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