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Original Article

The Efficacy of Pure Natural Repellents on Rat Responses Using Circular Open Field

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Abstract

Rodent infestation is considered as one of the major pest problems since it is not only acting as a health hazard, but also causes damages to household, agricultural fields and even transportation business. The use of rodenticides is a common approach to rodent control, however, most rodenticides are poisonous to man. Therefore, using natural extracts as a rat repellent may be a better alternative. In the current study, various natural extracts were experimented whether they could repel a rat when testing in the behavioral model, a circular open field. The tested substances were wintergreen oil, chilli, peppermint oil, bergamot oil and geranium oil either being applied as singly or in combination. For the natural behavior of the rats in the apparatus, the rats had the highest activity rate during the beginning of the nocturnal phase (light offs) as shown by total number of line crossed during 30 min. Therefore, this period was used for testing the efficacy of rat repellents. We found that the number of visit to the tested core and the time rat spent near the tested core significantly lowered compared to blank control. We can therefore conclude that these natural extracts can repel the rats as determined by rat's behaviors in the circular open field. However, more studies need to be done to see whether these extracts are of practical in real environment.

Keywords: Circular open field, natural repellent, rat

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บทคัดย่อ

ประสิทธิผลของสารสกัดจากธรรมชาติในการไล่หนูเมื่อทดสอบด้วยอุปกรณ์ทดสอบพฤติกรรม Circular open field

สฤณี กลันทกานนท์ ทองทรง [1] สุวภรณ์ แดนดี [2] บุญฤทธิ์ ทองทรง [3] วิวัฒน์ ชวนะนิกุล [3]*

ปัญหาการรบกวนจากหนูเป็นปัญหาสำคัญ เนื่องจากหนูเป็นสัตว์ที่นอกจากจะเป็นปัญหาด้านสุขภาพแล้ว การรบกวนจากหนูก่อให้เกิดผลเสียหายต่อบ้านเรือน พืชผลการเกษตร ตลอดจนอุตสาหกรรมการขนส่ง การใช้ยาเบื่อหนูเป็นวิธีการควบคุมจำนวนประชากรหนูที่มีการใช้มากที่สุด แต่ข้อเสียของการใช้ยาเบื่อหนูคือความเป็นพิษต่อคน ดังนั้นการใช้สารอื่นโดยเฉพาะสารสกัดจากธรรมชาติในการไล่หนูจึงเป็นทางเลือกหนึ่งที่น่าสนใจ ในการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อทดสอบว่าสารสกัดจากธรรมชาติ ได้แก่ น้ำมันระกำ พริก น้ำมันสะระแหน่ น้ำมันมะกรูด และน้ำมันเจอเรเนียมมีคุณสมบัติในการไล่หนูหรือไม่เมื่อทดสอบในอุปกรณ์ทดสอบพฤติกรรม Circular open field เมื่อทำการศึกษาโดยการปล่อยให้หนูอยู่ในอุปกรณ์ทดสอบเป็นเวลา 24 ชั่วโมงพบว่า หนูมีความตื่นตัวมากที่สุดในช่วงเริ่มต้นของกลางคืน (เมื่อปิดไฟ) โดยดูจากจำนวนเส้นที่หนูข้ามในช่วงเวลา 30 นาที ดังนั้นจึงใช้ช่วงเวลานี้ในการทดสอบประสิทธิผลของสารสกัดต่างๆ ในการไล่หนู พบว่าหนูกลุ่มที่ได้รับสารทดสอบทุกชนิดมีจำนวนครั้งที่มาสัมผัสแกนทดสอบและใช้เวลาอยู่ในส่วนในของอุปกรณ์ทดสอบน้อยกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ การทดลองนี้สามารถสรุปได้ว่าสารสกัดทุกชนิดสามารถไล่หนูได้เมื่อดูจากผลการทดสอบใน Circular open field อย่างไรก็ตามควรจะได้มีการศึกษาถึงการนำไปใช้ต่อไป

คำสำคัญ: อุปกรณ์ทดสอบ circular open field สารไล่หนู หนู

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Introduction

Rodent infestation is one of the major pest problems since it is not only considered as a health hazard but also causes damages to household, agricultural fields and even transportation business. On a ship, rats can cause extensive damage to cargo and food and rat-droppings contain organism that produce intestinal diseases (WHO, 1988). It has been estimated that the total cost of destruction by rats in the United States may be as high as $19 billion per year (Pimentel et al., 2005). In India, analysis of the information available on the damage and economic losses caused by rodents in various crop fields, horticulture and forestry, poultry farms, and rural and urban dwellings and storage facilities showed that chronic damage ranging from 2% to 15% persisted throughout the country and up to 100% loss of the field crop was not rare in severe damage (Parshad, 1999). Several approaches to control rodent infestation are being used such as environmental, cultural, biological, mechanical and chemical methods. In a ship, mechanical method like trapping is a good method of keeping down the rat population in which snap trap is more effective and practical than cage type (WHO, 1988). However, the use of chemicals like rodenticides is the common approach in South Asia (Parshad, 1999). The rodenticides can be classified into slow-acting and fast-acting rodenticides (WHO, 1988). The former such as warfarin and diphenadione are anticoagulants and must be ingested for several consecutive days before they become effective. The latter such as zinc phosphide and bormethalin are also known as acute rodenticides and often kill with a single dose. However, most rodenticides are poisonous to man and the effectiveness of rodenticides depends upon the selection of an appropriate compound and its formulation as bait. Moreover, the method and timing of application are needed to be considered. One problem of using bait is that if the rats have taken an initial non-fatal dose, it can discourage the rats from taking additional bait known as bait shyness. The chemical usages are not limited to rodenticides but chemical repellents like copper oxychloride, thiram, beta-nitrostyrene, cycloheximide and tribotyltin had been effectively tested in laboratory (Tigner, 1966; Parshad, 1999). Major factors that limit the usage of chemical repellent are possibly handling hazard and food contamination if apply on individual boxes or sacks containing food for human (Tigner, 1966). Due to possible chemical toxicity, the alternative like natural extracts should be considered. The interested substances are chilli, wintergreen oil, bergamot oil, peppermint oil and geranium oil as it had been reported that these substances had insect repellent properties. Lale (1992) had shown that powders prepared from ground chilli can repel the bruchid beetle or the cowpea weevil (*Callosobruchus maculates*). White et al. (2009) reported that wintergreen oil could kill bee (*Osmia cornifrons*) or bee's cleptoparasitic mite, *Chaetodactylus krombeini* Bakerbee, depending on concentration and duration of fumigation. Moreover, wintergreen and peppermint oils were found to be the two most effective essential oils at reducing attractiveness of the Japanese beetle (Youssef et al., 2009). In addition, larvicidal activity was shown with bergamot oil (Melliou et al., 2009) or peppermint oil (Ansari et al., 2000). In water buffaloes, Khater et al. (2009) reported that peppermint oil could be used to control lice and flies infestation as it contained lousicides and insect repellents activity. Geranium oil was shown to repel mosquitoes (*Ixodes ricinus*) (Jaenson et al., 2006), chigger (*Leptotrombidium chiggers*, larvae), the carrier of scrub typhus (Eamsobhana et al., 2009), and stable flies (*Stomoxys calcitrans*) (Hieu et al., 2010).

In order to evaluate the efficacy of rat repellents, there is a number of laboratory testings such as food acceptance test, barrier test and graded strip test (Weeks, 1959). In food acceptance test, the repellent is mixed with food and the effectiveness is based on the amount of food eaten by a rat in a period of time. Unfortunately, in this test, some chemicals were able to make the food unacceptable but were failed to repel the rat. In barrier test, hungry rats must be trained to gnaw through repellent-coated paper barrier and time required to penetrate the paper are recorded. In graded strip test, a rat has to gnaw through the paper strip coated with varied concentration of repellents to get to palatable food like peanuts which are placed under each strip and then the repellent activity is calculated based on numbers of obtained peanuts. For the latter two tests, the rats must be hungry and trained; moreover the instruments must be specially made and analyzed with care. The open field is a behavioral model usually used for analysis of locomotor activity or anxiety. In this test, the rats are allowed to explore freely in the apparatus for a period of time, then the number of line crossed or time spent in each segment of the apparatus are analyzed according to types of tests. Circular open field is also used for measuring locomotor activity in rats. In this study the circular open field was adapted in order to observe 24-hour activity of the rats exposed to various repellents.

The objectives of this study were, therefore, to observe the natural behavior of rats in the circular open field when exposed to various natural repellents and whether these repellents were as effective as seen in other species.

Materials and Methods

Animals: Adult male Wistar rats weighing 210-220 g at the beginning of the experiments were obtained from the National Laboratory Animal Center, Mahidol University (NLAC-MU), Thailand. All animals were housed 2 per cage and maintained at 25±2°C on 12-hrs light/dark cycle with lights on at 0600 am and given standard rat chow and water *ad libitum*. All procedures were done under the approval of the Animal Use Committee, Faculty of Veterinary Science, Chulalongkorn University.

Repellents: The repellents and cardboard paper were supplied by Interink Co. Ltd. They were wintergreen oil+chilli, wintergreen oil+peppermint oil, bergamot oil, wintergreen oil+peppermint oil+bergamot oil and bergamot oil+geranium oil, designated as F1-F5. These substances had been registered for patent number 0901002635. The repellents were freshly sprayed on the cardboard paper before being placed into the apparatus.

Behavioral model: The circular open field was constructed as previously described by Itoh et al. (1994) with some modification (Figure 1). The apparatus was an aluminum tub-shaped enclosed with a circular base 75 cm in diameter. The top opening had a diameter of 85 cm and the wall had a height of 85 cm. On the bottom, two concentric circles with diameters of 25 and 50 cm were drawn resulting in 3 circular fields, inner, middle and outer zones. In the middle of the inner part, a stainless rod with the diameter of 1 cm was installed for the application of repellent-paper roll. The middle zone and the outer zone were further divided into eight and sixteen equal parts by lines, respectively.

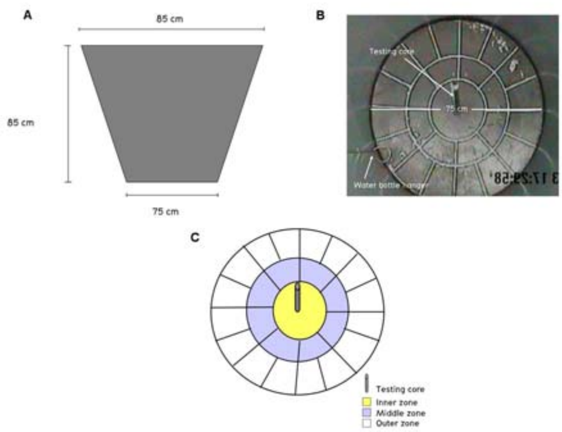

Figure 1. The circular open field, an aluminum tub-shaped apparatus with a based-diameter of 75 cm, an opening-diameter of 85 cm with a height of 85 cm (A). The water bottle hanger and a metal testing core are installed for water dispensing during 24-hour test and for cardboard paper placer, respectively (B). The base of the apparatus was divided into 3 zones; inner zone, middle zone and outer zones as shown in C.

Behavioral test: The test composed of a 3-day training and the tests were done on the following day and then 7 days later. This training period was done in order to reduce the fear and anxiety of the rats when exposed to a new environment. This was done by placing each pair of rat from the same cage into the apparatus and allowing them to freely explore the open field arena for 30 minutes for 3 consecutive days. The next day, each pair of rats was placed in the apparatus at 06.00 pm and their behaviors were observed for 24 hrs using closed circuit video recorder for further analysis. During this time, rats had free access to water. Seven days later, each pair of rats was exposed to the same repellent or control (blank). The behavioral analysis was done every six hours for 30 min, at 06.00-06.30 pm, 12.00-12.30 am, 06.00-06.30 am and 12.00-12.30 pm designated as P1, P2, P3 and P4, respectively. The parameters were numbers of visits and time spent in the inner, middle and outer zones. If the tested substances could repel the rats, the numbers of visits and time spent would be fewer in the inner zone and higher in the outer zone. Moreover, the total number of line crossed during 30 min was also recorded and considered as locomotor activity of the rats. In order to test the efficacy of potential repellent, the fear of novelty as being exposed to a new environment was reduced by the training period and by placing the rats in pair as rats are social animals. Because it had been shown that memory could last for at least 5 days following learning (Rossato et al., 2006), a 7 day post-exposure was then used to test whether the rats could remember the substances. For each substance, 3 pairs of rat were used and the behavioral data were analyzed and scored by 2 experimenters blinded to the experiment.

Statistical analysis: Data are presented as mean+SE. Two-way analysis of variance (ANOVA) with treatments or periods (P1-P4) as the independent factor and days (day 0 and 7) as the dependent factor were used. In order to test the significant effect of treatments or periods of time in the same day, data were analyzed by one-way ANOVA followed by the Duncan post hoc test. In all cases, a value of $p < 0.05$ was considered significant.

Results

The effect of time on rat behavior in the circular open field: In this study, rats were placed in the circular open field for 24 hrs (06.00 pm–05.00 pm of the following day) then the numbers of visits, time spent in the inner zone, middle zone and outer zone of the apparatus and total number of line crossed during 30 min were counted, which were done for 4 periods (P1, P2, P3 and P4). The same procedures were repeated again on day 7.

For the numbers of visits, the two-way analysis of variance (ANOVA) with the periods of time (P1-P4) and days (day 0 and 7) as the variables were used. There was no significant effect of days (p = 0.6406) nor an interaction of day x period of times (p = 0.3362). However, we found that different period of time had significant effect on numbers of visits (p = 0.0255) in that rats visited the testing core more frequent during 06.00-06.30 pm (P1) on both day 0 and day 7 (Figure 2).

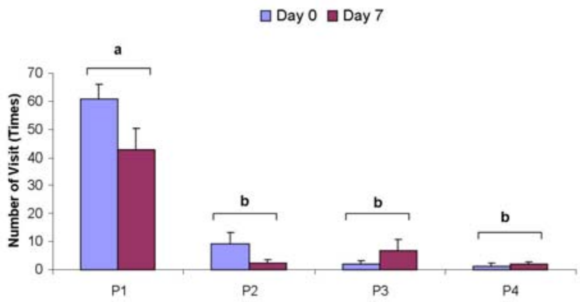

Figure 2. The effect of time on numbers of visits to the testing core in circular open field, Data presented as mean±SEM, different letters denoted significant difference at p<0.05, ANOVA followed by Duncan's multiple range test, n= 6.

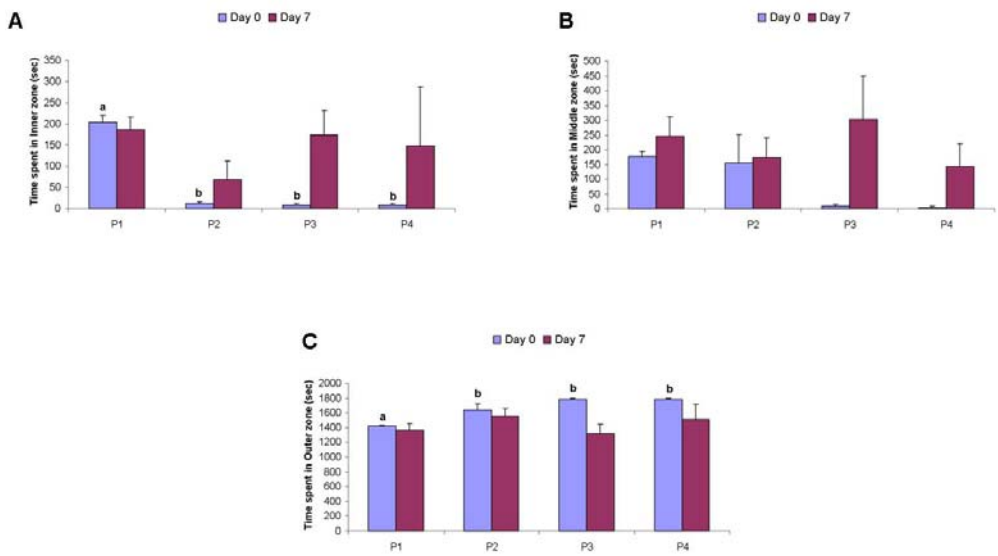

Figure 3. The effect of time on the time the rats spent in each part of the circular open field, A. Inner zone, B. Middle zone and C. Outer zone. Data presented as mean±SEM, different letters denoted significant difference at p<0.05 between each time point (P1-P4) in the same day (Day 0 and Day 7), ANOVA followed by Duncan's multiple range test, n= 6.

For the time spent in each part of the apparatus, there was no significant effect of days on the time spent in the inner zone (p = 0.9240), the middle zone (p = 0.4053) nor the outer zone (p =

0.5046). Similarly, there was no significant effect of different periods of time on the time spent in the inner zone (*p* = 0.2143), the middle zone (*p* = 0.4673) nor the outer zone (*p* = 0.3377). Moreover, there was no significant effect of interaction of day x period of times on the time spent in the inner zone (*p* = 0.4454), middle zone (*p* = 0.5255) nor the outer zone (*p* = 0.4519). However one-way ANOVA revealed that on day 0, the rats spent more time in the inner zone and less time in the outer zone during 06.00-06.30 pm (P1) than other periods ($p < 0.0001$ and p = 0.0009 for inner and outer zone, respectively). The time rats spent in the middle zone tended to be different across times (*p* = 0.0712). On day 7, periods of time had no effect on time that the rat spent in the inner (*p* = 0.8703), middle (*p* = 0.6126) and outer zone (*p* = 0.7229) (Figure 3).

For the total number of line crossed during 30 min, there was no significant effect of days (day 0 vs. day 7, *p* = 0.8727) nor the interaction of days and periods of time (*p* = 0.1277). However, different periods of time had significant effect on the numbers of lines crossed during 30 min ($p < 0.0001$). One way ANOVA revealed that the rats had more activity as indicated by higher numbers of lines crossed in 30 min during 06.00-06.30 pm (P1) than other periods ($p < 0.0001$ and $p < 0.0001$ for day 0 and day 7, respectively) as shown in Figure 4.

All of above indicated that rats had highest activity for the first 30 min in the circular open field as shown by the numbers of visits and the total numbers of lines crossed. Since time spent in each part of the apparatus could be affected by lack of activity of the rat and could have led to misinterpretation of the data, the collected parameters from the time between 06.00-06.30 pm were selected and analyzed in the further experiments to test the efficacy of various repellents.

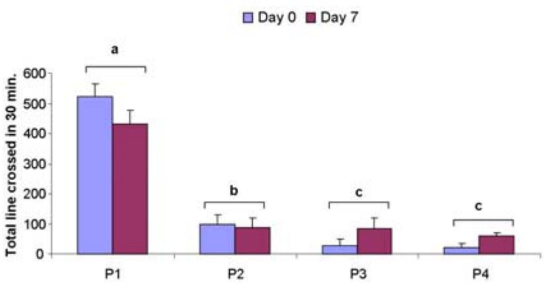

Figure 4. The effect of time on the total numbers of lines crossed during 30 min in the circular open field. Data presented as mean±SEM, different letters denoted significant difference at $p < 0.05$ between each time point (P1-P4) in the same day (Day 0 and Day 7), ANOVA followed by Duncan's multiple range test, n= 6.

The effect of various repellents on rat behavior in the circular open field: In this study, the rats were exposed to various repellents: blank cardboard paper, or the cardboard paper sprayed with either wintergreen oil+chili (F1), wintergreen oil+peppermint oil (F2), bergamot oil (F3), wintergreen oil+peppermint oil+bergamot oil (F4) or bergamot oil+geranium oil (F5) in the circular open field for 24 hrs (06.00 pm–05.00 pm of the following day) then the numbers of visits and time spent in the inner zone, middle zone and outer zone of the apparatus were counted during 06.00-06.30 pm. The same procedures were repeated again on day 7.

For the numbers of visits, the two-way analysis of variance (ANOVA) with treatments and days (day 0 and 7) as the variables were used. There was no significant effect of days (*p* = 0.2909) nor an interaction of treatments x days (*p* = 0.8406). However, we found that different repellents had significant effect on the numbers of visits (*p* = 0.0227) in that rats visited the testing rod less frequent in all repellents compared to control on both day 0 and day 7 (*p* = 0.0227). One way ANOVA revealed that on day 0, all repellents had a significant effect on the numbers of visits ($p < 0.0001$) and the rats exposed to F2, F3 and F4 had fewer visits than F1 and F5. Similarly, on day 7, all repellents had a significant effect on the numbers of visits ($p < 0.0001$) and the rats exposed to F2, F3, F4 and F5 had fewer visits than F1 (Figure 5).

For the time spent in each part of the apparatus, the two-way analysis of variance (ANOVA) with treatments and days (day 0 and 7) as the variables were used. There was no significant effect of days on the time spent in the inner zone (*p* = 0.6271) nor the outer zone (*p* = 0. 3201). Similarly, there was no significant effect of the interaction between treatments and days on the time spent in the inner zone (*p* = 0.6039) nor the outer zone (*p* = 0.1828). However, there were significant effects of days and the interaction between treatments and days on the time spent in the middle zone (*p* = 0.0007 and 0.0007, respectively) in that on day 7, F3 spent more time in the middle zone when compared to day 0 (Figure 6B).

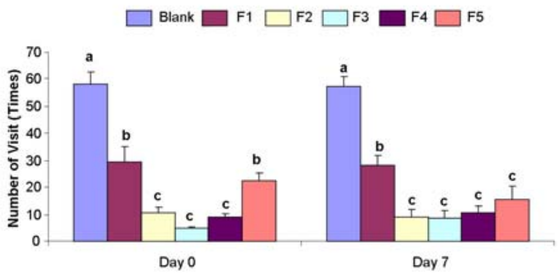

Figure 5 The effect of various rat repellents on numbers of visits to the testing core in the circular open field, Data presented as mean±SEM, different letters denoted significant difference at $p < 0.05$ between substances in the same day (day 0 and day 7), ANOVA followed by Duncan's multiple range test, n= 6 in each treatment.

Moreover, we found that repellents had a significant effect on the time the rats spent in the inner zone (*p* = 0.0197), middle zone ($p < 0.0001$) and the outer zone (*p* = 0.0079). One-way ANOVA revealed that the rats spent less time in the inner zone when exposed to repellents compared to the control on both day 0 ($p < 0.0001$) and day 7 ($p < 0.0001$) (Figure 6A).

The time the rats spent in the middle zone was significant only on day 0 ($p < 0.0001$) in that the rats exposed to F2, F3 and F4 spent less time than the control and F1 and F5 spent less time than F1 but not different from the control but more than F2, F3 and F4. This effect was not seen on day 7 ($p = 0.1289$) (Figure 6B). For the time spent in the outer zone, the rats exposed to all repellents spent more time in the outer zone than the control ($p < 0.0001$) on day 0. On day 7, it tended that the rats exposed to repellents would spend more time in the outer zone than the control ($p = 0.0724$) (Figure 6C).

Moreover, it was worth looking at the cardboard after 24-hrs exposed to the rats in the circular open field, it was quite cleared that the cardboards sprayed with F1 were still intact compared to the others (Figure 7B). For other groups, the bitten and chewed marks varied between each rat; however, we noticed that the F4- and F5- treated cardboards were less likely to be destroyed especially when compared to blank.

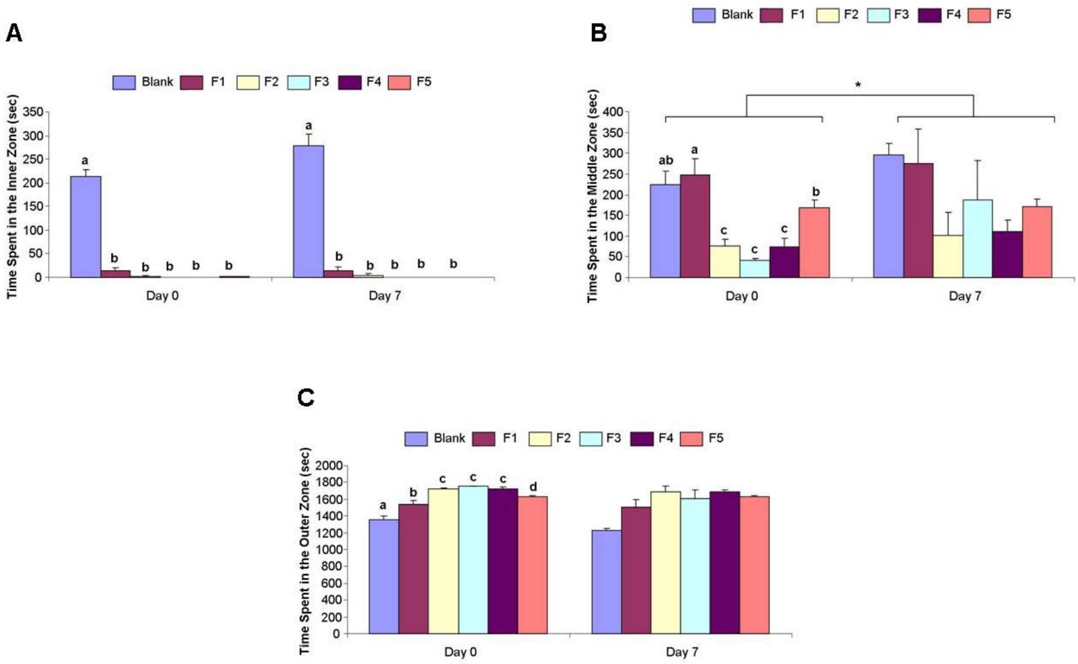

Figure 6. The effect of rat repellents on the time the rats spent in each part of the circular open field, A. Inner zone, B. Middle zone and C. Outer zone. Data presented as mean+SEM, different letters denoted significant difference at $p < 0.05$ between each substance (blank, F1-F5) in the same day (Day 0 and Day 7), ANOVA followed by Duncan's multiple range test. * denoted significant different at $p < 0.05$ between day 0 and day 7, two-way ANOVA, n= 6 in each treatment.

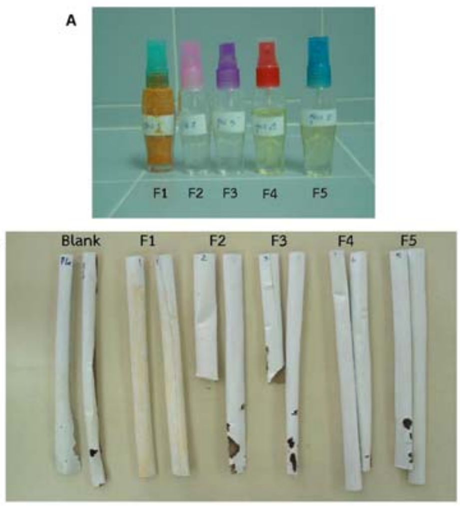

Figure 7A. The rat repellents **B**. An example of paper cardboard sprayed with various repellents after 24-hour-exposed to the rats in the circular open field.

Discussion

Rodent infestation is considered as one of the health hazards; therefore, many methods have been adopted such as the used of trapping, rodenticides or repellents. However, there are limitations to these methods including chemical toxicities. The alternative to chemicals was therefore of interested since there were numbers of reports claiming that some natural extracts like winter green oil, chilli, peppermint oil, bergamot oil or geranium oil contained insect repellent properties (Lale, 1992; Ansari et al., 2000; Jaenson et al., 2006; Eamsobhana et al., 2009; Khater et al., 2009; Melliou et al., 2009; White et al., 2009; Youssef et al., 2009; Hieu et al., 2010). In this study, these natural extracts were tested whether they were potential rat repellents using modified circular open field. The circular open field was modified so that the test substances could be installed in the middle of the apparatus (testing core). In this test, the numbers of visits to the testing core and time the rats spent in each part of the apparatus were analyzed. If the repellents were able to repel the rats then the numbers of visits to the testing core and the time the rats spent in the closed perimeter to the testing core (inner zone) would decreased and the time the rats spent away from the testing core (outer zone) would increased. Additionally, if the rats could remember the repellents, then the analyzing parameters would be differed on day 7.

In order to evaluate the efficacy of repellents in the circular open field, the natural behavior was observed for 24 hours from 18.00 hr. (the beginning of the dark phase) to 17.00 hr. of the following day. Then the numbers of visits to the testing core and the time the rats spent in each part of the apparatus were analyzed every 6 hours for 30 min. during 06.00-06.30 pm, 12.00-12.30 am, 06.00-06.30 am and 12.00-12.30 pm designated as P1-P4. We found that the rats visited the testing core more frequent and spent more time in the inner zone and less time in the outer zone during the first 30 min (P1) after being placed in the apparatus. However, the time spent in each part of the apparatus i.e. inner, middle and outer zones may be confounded by the lack of the rat's activity, then the numbers of total line crossed during 30 min. were taken into account. Similarly, the numbers of total line crossed was highest during the first 30 min (P1). It was thus likely that the rats had the highest activity during the first 30 minutes in the apparatus as it was the beginning of the nocturnal phase (lights off) of the rat's circadian rhythm. During the nocturnal phase of the circadian cycle in terms of natural behavior, rats were usually active, inquisitive and more responsive compared to diurnal phase (lights on). Therefore, in order to test the efficacy of rat repellents in the circular open field, the time of highest activity (06.00-06.30 pm) was used for analysis.

When the rats were exposed to potential rat repellents i.e. wintergreen oil+chilli, wintergreen oil+peppermint oil, bergamot oil, wintergreen oil+peppermint oil+bergamot oil or bergamot oil+geranium oil, we found that the numbers of visits to the testing core and the time spent in the inner zone were lower in all repellent groups compared to blank on both day 0 and day 7 indicating that the rats were most likely trying to avoid the closed contact to these substances. Although previous studies have claimed that these substances were potential insect repellents (Lale, 1992; Ansari et al., 2000; Jaenson et al., 2006; Eamsobhana et al., 2009; Khater et al., 2009; Melliou et al., 2009; White et al., 2009; Youssef et al., 2009; Hieu et al., 2010), this current study is the first report claiming these substances as potential rat repellents. Since the rats were left exposed to the substance for 24 hours in the circular open field, it was then worth looking at the cardboards sprayed with various substances. We found that although the analyzed parameters showed no different in repellent activity, the cardboards somehow differed in the degree of tearing and chewing (Figure 7B). Inconsistent degree of cardboard destruction were found, which may be accounted by the rat's behaviors; some may be more tolerant to the substances' odor than others and resulting in various results. However, we found that the cardboards sprayed with wintergreen oil+chilli or wintergreen oil+peppermint oil+bergamot oil consistently showed no sign of tearing or chewing; however, some footprints may be evident. For the cardboard sprayed with bergamot oil+geranium oil, there was less tearing or chewing than the cardboards sprayed with wintergreen oil+peppermint oil, bergamot oil and blank. It is thus likely that for a longer period of exposure both behavioral data in the circular open field and the cardboard appearance after 24-hour exposure should be taken into account as these potential repellents may be further applied on packaging and shipping supplies to prevent rat's destruction. Moreover, it should be noted that in terms of application, the wintergreen oil+chilli may be a little hard to be applied as a spraying product due to its viscosity.

From these data, we can conclude that these natural extracts i.e. chilli, wintergreen oil, bergamot oil, peppermint oil and geranium oil can repel the rats as shown by the lower numbers of visits to the tested core and less time spent near the tested core seen in the circular open field. However, more studies need to be done like stability and period of protection to see whether these extracts are of practical in real environment.

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