Original Article

Evaluation of Cardboard Coated with Natural Substances in Combination with Ink on Rat Repellency

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Abstract

An effective repellent for rodent-proof cardboard-container is considered interesting especially in packaging and transport business as rat usually gnaws through the container, resulting in food deterioration or goods loss. Rodenticide usage is a common approach for rodent control; however, most rodenticides are poisonous to man as well as to the rats. Therefore, preferably the usage of non-toxic rat repellent is an interesting alternative. In this study, various natural extracts, i.e. wintergreen oil, chilli, peppermint oil, bergamot oil and geranium oil, which had been shown to repel rats when apply singly or in combination, were mixed with ink, coated on cardboard before being placed in circular open field with rats for 24 hours. In this study, two forms of application were used which were coating the whole cardboard and coating the cardboard as a striped-like manner. We found that the number of visits to the tested core and the time the rats spent near the tested core were significantly lower in the rats exposed to the ink and bergamot oil formula when compared to those exposed to blank cardboard, which was the control group, in both forms of application. However, the behaviors of the rats exposed to the ink, chilli and wintergreen oil formula and the ink, peppermint oil and wintergreen oil formula were not different from those of the control group. We can, therefore, conclude that ink in combination with bergamot oil alone, with bergamot oil, wintergreen oil and peppermint oil, and with bergamot oil and geranium oil have a potential for using as a rat repellent when applied on transporting packages.

Keywords: bergamot oil, chilli, circular open field, geranium oil, peppermint oil, rat repellent, wintergreen oil

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บทคัดย่อ

การประเมินคุณสมบัติในการไล่หนูของกระดาษเคลือบด้วยสารสกัดจากธรรมชาติผสมกับหมึกพิมพ์

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

สำหรับอุตสาหกรรมการขนส่งนั้น การมีวัตถุภัณฑ์ที่สามารถป้องกันการกัดทำลายจากหนูเป็นสิ่งที่น่าสนใจ เนื่องจากในระหว่างการขนส่งพบว่าหนูมักจะกัดทำลายกล่องเป็นผลให้เกิดความเสียหายกับผลิตภัณฑ์ภายใน การใช้ยาเบื่อหนูเป็นวิธีการควบคุมจำนวนประชากรหนูวิธีหนึ่ง แต่พบว่าสารที่ใช้ในการเบื่อหนูมักมีความเป็นพิษสูงทั้งต่อคนและหนู ดังนั้นการใช้สารไล่หนูที่ไม่เป็นพิษต่อคนจึงเป็นทางเลือกที่น่าสนใจ การศึกษานี้ทำการทดสอบสารสกัดจากธรรมชาติที่มีรายงานว่ามีประสิทธิภาพในการไล่หนูเมื่อทดสอบด้วยอุปกรณ์ทดสอบ circular open field ได้แก่ น้ำมันระกำ พริก น้ำมันสาระแหน่ น้ำมันมะกรูด และน้ำมันเจอเรเนียม โดยทำการผสมสารสกัดเหล่านี้ในหมึกพิมพ์แล้วนำไปเคลือบบนกระดาษกล่อง ก่อนจะนำไปวางในอุปกรณ์ทดสอบกับหนู เป็นเวลา 24 ชั่วโมง การทดสอบแบ่งลักษณะการเคลือบเป็น 2 รูปแบบ ได้แก่ การเคลือบบนกระดาษทั้งแผ่น และการป้ายเป็นแถบ คล้ายกับที่ต้องการใช้จริง ผลการทดสอบพบว่าในหนูที่ทดสอบกับหมึกพิมพ์ที่มีส่วนประกอบของน้ำมันมะกรูดนั้น หนูเข้ามาสัมผัสที่แกนทดสอบและใช้เวลาอยู่ใกล้กับแกนทดสอบน้อยกว่ากลุ่มควบคุมที่มีแต่หมึกเพียงอย่างเดียว ในขณะที่หนูที่ทดสอบกับหมึกพิมพ์ที่ผสมกับน้ำมันระกำกับพริก และน้ำมันสาระแหน่กับน้ำมันระกำ มีพฤติกรรมไม่ต่างจากหนูกลุ่มควบคุม อาจสรุปได้ว่าหมึกพิมพ์ผสมน้ำมันมะกรูด หรือน้ำมันมะกรูดกับน้ำมันระกำกับน้ำมันสาระแหน่ หรือน้ำมันมะกรูดกับเจอเรเนียม มีแนวโน้มที่จะสามารถนำไปใช้เป็นส่วนผสมของสารไล่หนูเพื่อใช้ในอุตสาหกรรมการขนส่งได้

คำสำคัญ: น้ำมันมะกรูด พริก อุปกรณ์ทดสอบ circular open field เจอเรเนียม น้ำมันสาระแหน่ สารไล่หนู น้ำมันระกำ

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Introduction

An effective repellent for rodent-proof cardboard-container is considered interesting especially in packaging and transport business as rat usually gnaws through the container, resulting in food deterioration or goods loss. 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). A similar finding was also reported in India (Parshad, 1999). Several approaches are being used to control rodent infestation such as environmental, cultural, biological, mechanical and chemical methods. For shipping, mechanical methods like trapping is a good method of keeping down the rat population (WHO, 1988); however, it requires laboring approach. Rodenticide usage is more common. Another method is the use of chemical repellents like copper oxychloride, thiram, beta-nitrostyrene, cycloheximide and tribotyltin, which were tested in laboratory (Tigner, 1966; Parshad, 1999). However, rodenticides and chemical repellents are poisonous to man as well as to the rats. Moreover, a possible handling hazard and food contamination have to be taken into account if they are applied on individual boxes or sacks containing food for human (Tigner, 1966). Due to possible chemical toxicity that limits the usage of chemical repellent, an alternative like natural extracts should be considered.

In order to evaluate the efficacy of rat repellents, circular open field was adopted. Based on the explorative nature of rats, this behavioral model is usually used for monitoring locomotor activity and/or anxiety depending on selected parameters. Total number of line crossed during a limited time is usually used as an indicator of locomotor activity of the rat while time spent in each part of the apparatus is used as an indicator of anxiety. If the rat spends longer time in the inner zone of the maze, it generally implies as less anxiety. However, following a training period, the rats will be habituated to the apparatus resulting in less fear of a novel environment; therefore, the locomotor activity can be favorably measured with no emotional effect. During the testing period, the rats were allowed to explore freely in the apparatus installed with tested substances, then the time spent in each segment of the apparatus was

analyzed. Because of its shape, the circular open field allows easily measuring the rat's activities in term of perimeter. We proposed that if the rats avoided the tested substance by spending less time in a closer proximity and/or lesser number of visits to the tested core, then the tested substance could repel the rats.

Various natural extracts, i.e. wintergreen oil, chilli, peppermint oil, bergamot oil and geranium oil, had been proven to contain insect repellent property (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). Previously, we had demonstrated that various natural extracts, i.e. wintergreen oil, chilli, peppermint oil, bergamot oil and geranium oil, were possibly able to repel rats when observed under the circular open field behavioral task as determined by the duration or the number of visits to the tested substances (Kalandakanond-Thongsong et al., 2010). However, the uses of pure extract may be inapplicable in transportation business. Therefore, this study aimed to experiment the efficacy of cardboard coated with mixtures of these natural substances and ink as rat repellents in the circular open field.

Materials and Methods

Animals: Adult male Wistar rats weighing 210-220 g at the beginning of the experiment were obtained from the National Laboratory Animal Center, Mahidol University (NLAC-MU), Thailand. All animals were housed 2 per cage, maintained at 25±2°C on 12-hour light/dark cycle with lights on at 06.00 am and recieved 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: Cardboard sprayed with ink in combination with different repellents were supplied by Interink Co., Ltd. The repellents were mixed with ink and designated as F1-F5, which were ink + wintergreen oil + chilli, wintergreen oil + peppermint oil, bergamot oil, wintergreen oil + peppermint oil + bergamot oil and bergamot oil + geranium oil, respectively. These substances had been registered for patent number 0901002636. The coated cardboard was

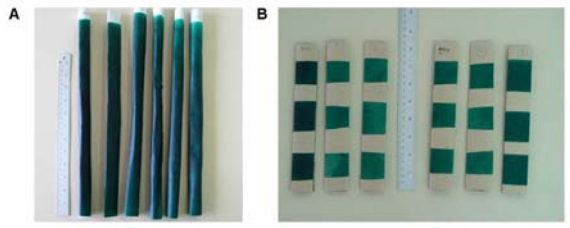

Figure 1 The coated cardboard sprayed with various repellents mixed with ink as a whole (A) and as a striped-like manner (B).

provided in 2 sets; for the first set, the whole cardboard was sprayed with the mixtures; for the second set, the cardboard was applied with the mixtures in a striped-like manner as it would be used in transportation business as shown in Figure 1.

Behavioral model: The circular open field was constructed according to Itoh et al. (1994) with some modifications as previously described (Kalandakanond-Thongsong et al., 2010) as shown in Figure 2. The apparatus was tub-shaped aluminum with a circular base, a top opening and a wall 75x85x85 cm in dimension. 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 a 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.

Behavioral test: The test was composed of 3-day training period and testing days which were done one day after the last training day and 7 days later. This training period was done in order to reduce the fear and anxiety of the rats when being exposed to a new environment. This was done by placing each pair of rats from the same cage into the apparatus and allowing them to freely explore the open field arena for 30 min for 3 consecutive days. The rats were placed in pairs in order to reduce fear/anxiety and as they were social animal. On the next day, each pair of rats was placed in the apparatus at 06.00 pm and their behaviors were observed for 24 hours, using closed circuit video recorder for further analysis. During this

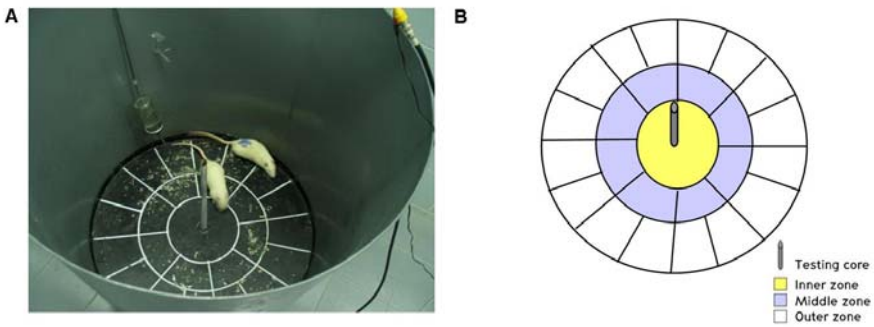

Figure 2 The circular open field, an aluminum tub-shaped apparatus with a dimension of 75 x 85 cm with an opening-diameter of 85 cm, the water bottle hanger and the metal testing core installed for water dispensing during 24-hour test and for cardboard placer, respectively (A). Division of the base of the apparatus into 3 zones: inner zone, middle zone and outer zone (B).

time, the rats had free access to water. Seven days later, each pair of rats was exposed to the same repellent or control (blank). The parameters were number of visits and time spent in the inner, middle and outer zones. If the tested substances could repel the rats, then the number of visits and time spent in the inner zone would be less and spend more in the outer zone. Because it had been shown that rat's memory could last for at least 5 days following learning (Rossato et al., 2006), 7 days post-exposure the rats were tested whether they could remember the substance. The behavioral data were analyzed during the first 30 min (06.00-06.30 pm), during which the rats had the highest activity in the maze, and scored by 2 experimenters blinded to the experiment.

Statistical analyses: Data are presented as mean ±SEM. One-way analysis of variance (ANOVA) was used to test the significant effect of treatments on the same day; in case of significance the Duncan post hoc test was adopted. In all cases, a value of $p<0.05$ was considered significant.

Results

The effect of various repellents mixed with ink (set 1) on rat behaviors in circular open field: In this study, rats were exposed to blank cardboard which served as the control group and cardboard sprayed with various repellents: mixtures of ink, wintergreen oil and chilli (F1); ink, wintergreen oil and peppermint oil (F2); ink and bergamot oil (F3); ink, wintergreen oil, peppermint oil and bergamot oil (F4); or bergamot oil and geranium oil (F5) in the circular open field for 24 hours (06.00 pm-05.00 pm. of the following day). Number 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.

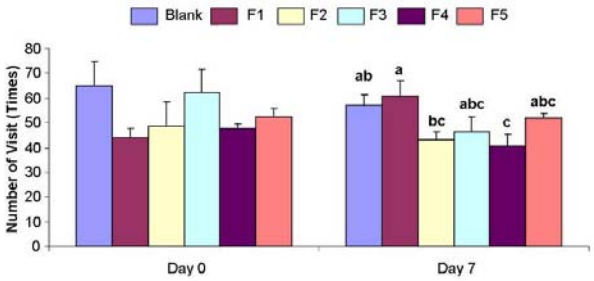

Figure 3 The effect of various repellents mixed with ink on the number of visits to the testing core in the circular open field, data presented as mean ± SEM, different letters denoting significant difference on the same day at $p<0.05$, ANOVA followed by Duncan's multiple range test, n=8 in each treatment.

For the number of visits on day 0, the rats visited the testing rod indifferently ($p=0.2229$). However, on day 7, the rats exposed to F4 had lesser number of visits than the control group and those exposed to F1; and the rats exposed to F2 had lesser visits than those exposed to F1 ($p=0.0319$) as shown in Figure 3.

For the time spent in each part of the apparatus on day 0, the rats exposed to every substances spent less time in the inner zone than the control group ($p=0.0190$; Fig 4A); while on day 7, only the rats exposed to F3 spent less time than the control group and those exposed to F1 ($p=0.0499$; Fig 4A). Moreover, we found that rats exposed to F2 and F4 tended to spend more time than the control group in the middle zone on day 0 ($p=0.0904$); and the rats exposed to F3 tended to spend more time than

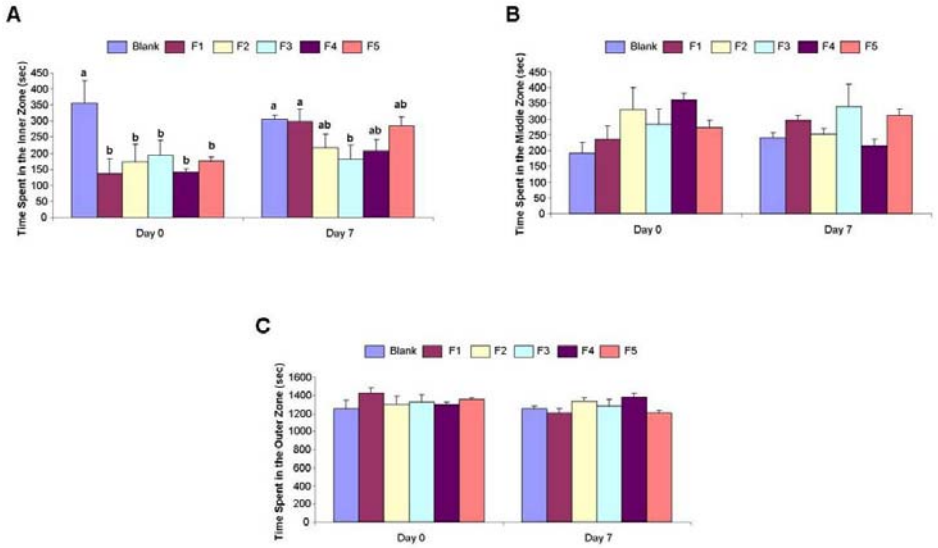

Figure 4 The effect of various repellents mixed with ink on the time the rats spent in each part of the circular open field, A. Inner zone, B. Middle zone and C. Outer zone, data presented as mean ± SEM, different letters denoting significant difference on the same day at $p<0.05$, ANOVA followed by Duncan's multiple range test, n=8 in each treatment.

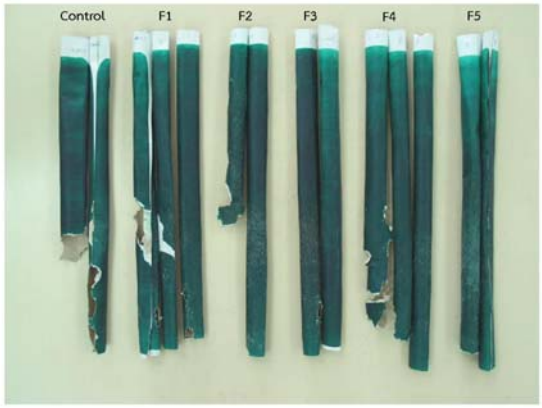

Figure 5 The example of cardboard sprayed with ink mixed with various repellents after 24-hour exposed to the rats in the circular open field.

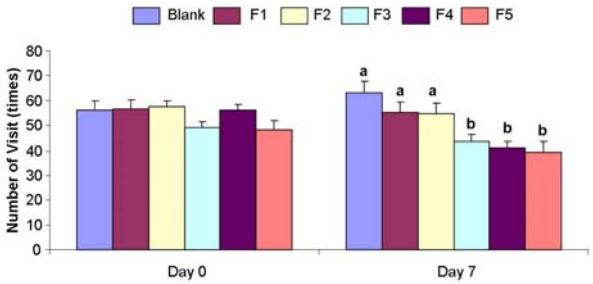

Figure 6 The effect of various repellents mixed with ink in a stripe-like manner on the number of visits to the testing core in the circular open field, data presented as mean + SEM, different letters denoting significant difference on the same day at $p<0.05$, ANOVA followed by Duncan's multiple range test, n=12 in each treatment.

those exposed to F4 in the middle zone but not different from the control group on day 7 (p=0.0933) as shown in Fig 4B. For the time the rats spent in the outer zone, there was no significant difference on day 0 between each group, but the rats exposed to F4 tended to spend more time in this area than those exposed to F1 but not different from the control group on day 7 (p=0.0724) as shown in Fig 4C. When we checked the coated paper, we noticed that the cardboard coated with F3 and the cardboard coated with F5 had less bitten and scratch marks than the others (Fig 5).

The effect of various repellents mixed with ink (set 2) on rat behaviors in circular open field: For the number of visits, there was no significant difference on day 0 (p=0.1220); however, on day 7, the rats exposed to F3, F4 and F5 had lesser number of visits than the control group and those exposed to F1 and F2 (p=0.0319) as shown in Fig 6.

For the time spent in each part of the apparatus, on day 0, the rats exposed to F3 and F4 spent less time in the inner zone than the control group (p=0.0093; Fig 7A); while the time spent in the inner zone of those exposed to F1, F2 and F5 were not different from the control group. On day 7, the rats exposed to F3 tended to spend less time in the inner zone than the control group and those exposed to F1 and F2 (p=0.0651; Fig 7A). In the middle zone of the maze, there was no significant difference between each group on day 0 (p=0.9353). Interestingly, we found that the rats exposed to F5 spent less time in the middle zone than the control group and those exposed to F1, F2 and F4; but those exposed to F3 spent lesser time than those exposed to

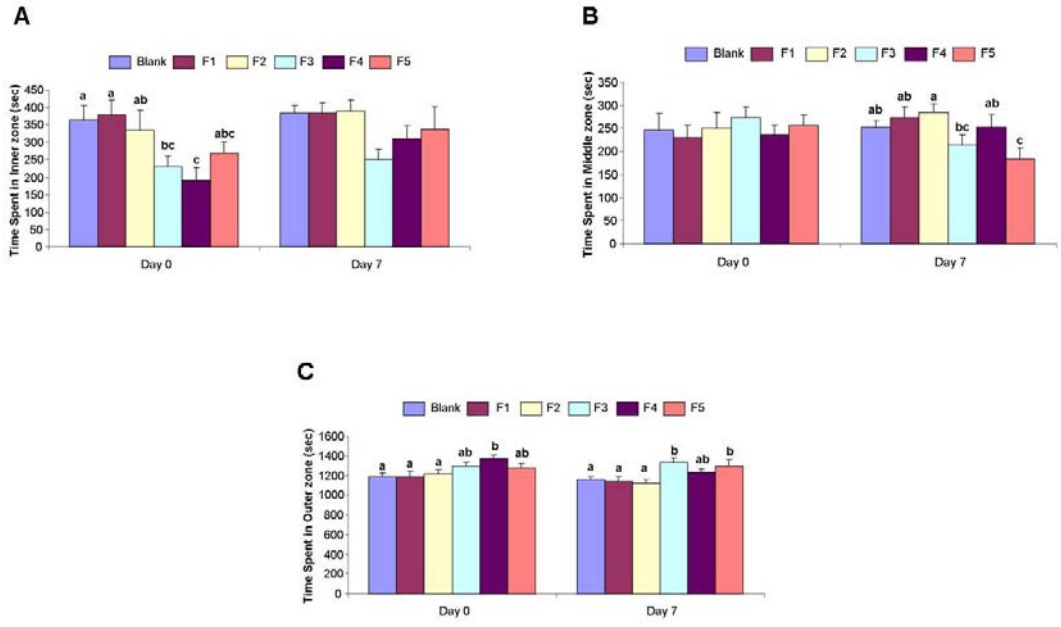

Figure 7 The effect of various repellents mixed with ink in a stripe-like manner on the time the rats spent in each part of the circular open field, A. Inner zone, B. Middle zone and C. Outer zone. Data presented as mean+SEM, different letters denoting significant difference on the same day at $p<0.05$, ANOVA followed by Duncan's multiple range test, n=12 in each treatment.

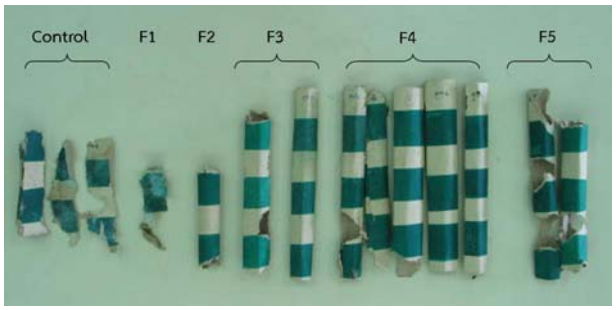

Figure 8 The example of cardboard sprayed with ink mixed with various repellents applied in a stripe-like manner after 24-hour exposed to the rats in the circular open field.

F2, but not different from the control group on day 7 (p=0.0029) as shown in Fig 7B. For the time the rats spent in the outer zone on day 0, only those exposed to F4 spent more time in this area than the control group and those exposed to F1 and F2 (p=0.0176); while on day 7, those exposed to F3 and F5 spent more time than the control group and those exposed to F1 and F2 (p=0.0022) as shown in Fig 7C.

Moreover, we noticed that the blank, F1 and F2 cardboard, after 24-hour exposure to the rats in the circular open field, was less intact compared to the others (Fig 8). Furthermore, only the coated part of F3 and F5 cardboard was not destroyed. Interestingly, the F4 cardboard was less likely to be destroyed especially when compared to the blank one.

Discussion

To control rodent infestation, rat repellent is one favorable method other than the uses of rodenticide. In order to avoid chemical toxicities, natural extracts are therefore the alternatives. Previously, we had discovered that the pure natural extracts of winter green oil, chilli, peppermint oil, bergamot oil and geranium oil contain rat repellent properties when tested in the laboratory using behavioral model (Kalandakanond-Thongsong et al., 2010). In this study, these natural extracts were mixed with ink and tested in the circular open field to evaluate whether they had potential for using in transportation business. This study was divided into 2 sets of experiment; firstly, the mixtures were coated on the whole piece of cardboard; secondly, the mixtures were coated in a striped-like manner as it would be applied on a package.

The data revealed that the mixture of ink, wintergreen oil and chilli (F1) failed to repel the rats as the number of visits and the time the rats spent in a closed proximity to the testing core were not different from the control group in both sets of experiment. Moreover, the cardboard coated with F1 was almost completely chewed and torn suggesting that it was not applicable. This finding was rather intriguing since this formula as a pure extract had been proved to repel rats (Kalandakanond-Thongsong et al., 2010). The mixture of ink, wintergreen oil and peppermint oil (F2) was probably a better candidate; however, it may require a higher amount or a higher concentration of the extract as the number of visits and the time the rats spent in the inner zone were less than the control group only in the first set of experiment, where there was more mixture applied. For the mixture of ink and bergamot oil (F3), we found that the number of visits and the time the rats spent closed to the substance were significantly less than the control group in both sets of experiment suggesting that this formula might still contain rat repellent activity. The results of the rats exposed to F4, ink, wintergreen oil, peppermint oil and bergamot oil, were somewhat similar the results of those exposed to F3 in that the number of visits and time spent in the inner zone were less than the control group. For the mixture of ink, bergamot oil and geranium oil (F5), we found that only on day 7 of the second set of experiment the rat avoided the substance as shown by the lower number of visits and lesser time spent in middle zone and longer time in outer zone. It was, therefore, likely that the formulas that contained bergamot oil still preserved rat repellent property. Interestingly, if the 24-hour exposed cardboard was to be taken into account for repellent activity, F3 may be a better candidate as it was destroyed less than the others in both sets of experiment. For F5, the cardboard was almost free from chewing and tearing in the first set of experiment; however, in the second set of experiment, the rats chewed on the uncoated area. This finding suggested that the higher concentration or the more area applied by the substance might be required for better protection. For F1 and F2, we found that these formulas could not protect the cardboard from destruction by the rats. This finding was rather intriguing since we reported earlier that F1 and F2 in the form of pure extract could repel rats (Kalandakanond-Thongsong et al., 2010). We speculated that the problem for F1, which contained chilli, was the incompatibility between ink and chilli as the extract was quite viscous.

From these data, we can conclude that these natural extracts, i.e. chilli, wintergreen oil, bergamot oil, peppermint oil and geranium oil, after being mixed with ink, only the formula containing bergamot oil can repel the rats as shown by the lowered number of visits to the tested core and the less time spent near the tested core seen in the circular open field. However, more studies should be done to test the stability and protection period when applied to transporting packages.

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