สัญญาเลขที่ **RMU5080036**

รายงานวิจัยฉบับสมบูรณ์

โครงการ การสร้างอุโมงค์ลมแบบสองทิศทางที่มีระบบนับจำนวนแมลงวัน เพื่อทดสอบกลิ่นที่เหมาะสม ในการใช้เป็นเหยื่อล่อแมลงวันบ้าน *Musca domestica* และแมลงวันหัวเขียว *Chrysomya megacephala* และการทดสอบกลิ่นดังกล่าวต่อแมลงวันทั้งสองชนิดในภาคสนาม

ผู้วิจัย นายคม สุคนธสรรพ์

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและ
สำนักงานคณะกรรมการการอุดมศึกษา

กิตติกรรมประกาศ

ขอขอบพระคุณสำนักงานกองทุนสนับสนุนการวิจัย และสำนักงานคณะกรรมการการอุดมศึกษา ซึ่งเป็นผู้สนับสนุนการวิจัยในโครงการนี้ ขอขอบคุณ ศาสตราจารย์ ดร. พงษ์ศักดิ์ อังกสิทธิ์ อธิการบดี มหาวิทยาลัยเชียงใหม่, รองศาสตราจารย์ นายแพทย์นิเวศน์ นันทจิต คณบดี คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่, รองศาสตราจารย์ ดร. อุดม ชัยทอง หัวหน้าภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ที่อนุญาตและสนับสนุนการทำวิจัยครั้งนี้

ขอขอบคุณ รองศาสตราจารย์ ดร. กาบแก้ว สุคนธสรรพ์ ที่เป็นผู้สนับสนุนการวิจัย ทำให้งานวิจัยครั้งนี้สำเร็จลุล่วงไปด้วยดี ขอขอบคุณคณะผู้ร่วมงานวิจัยดังนี้ คุณกิตติคุณ หมู่พยัคฆ์, อ.ดร. นพวรรณ บุญชู, อ. สรวิชญ์ อุปคุตม์, อ.ดร. รัชฎาวรรณ เงินกลั่น, นส. ธันวดี คล่องแคล่ว, คุณสมศักดิ์ เปียงใจและเจ้าหน้าที่ภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่, รศ.ดร. อรวรรณ ชัยลภากุล ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, อ. จิระ ปรังเขียว ภาควิชาภูมิศาสตร์ คณะสังคมศาสตร์ มหาวิทยาลัยเชียงใหม่, Prof. Kim N. Irvine, Department of Geography and Planning Department and Center for Southeast Asia Environment and Sustainable Development, Buffalo State, State University of New York, USA, Associate Professor Dr. Roy C. Vogtsberger (Department of Biology, Midwestern State University, USA), Dr. Hiromu Kurahashi (Department of Medical Entomology, The National Institute of Infectious Diseases, Japan)

บทคัดย่อ

รหัสโครงการ RMU5080036

ชื่อโครงการ การสร้างอุโมงค์ลมแบบสองทิศทางที่มีระบบนับจำนวนแมลงวัน เพื่อทดสอบกลิ่นที่เหมาะสม ในการใช้เป็นเหยื่อล่อแมลงวันบ้าน *Musca domestica* และแมลงวันหัวเขียว *Chrysomya megacephala* และการทดสอบกลิ่นดังกล่าวต่อแมลงวันทั้งสองชนิดในภาคสนาม

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ระยะเวลาโครงการ 3 ปี (25 มิถุนายน 2550 ถึง 25 มิถุนายน 2553)

เนื้อหางานวิจัย

แมลงวันบ้านและแมลงวันหัวเขียว *Chrysomya megacephala* เป็นแมลงวันที่มีความสำคัญทางการแพทย์ที่พบมากในประเทศไทย ตัวเต็มวัยเป็นพาหะนำเชื้อโรคหลายชนิดมาสู่มนุษย์ ก่อความรำคาญต่อการดำรงชีวิต ส่วนตัวอ่อนแมลงวันทำให้เกิดโรคหนอนแมลงวันทั้งในมนุษย์และสัตว์ การหาวิธีการควบคุมปริมาณแมลงวันจึงเป็นสิ่งที่จำเป็น การศึกษาครั้งนี้มีวัตถุประสงค์หลัก 3 ประการคือ (1) ออกแบบและสร้างอุโมงค์ลมแบบสองทิศทางที่มีระบบนับจำนวนแมลงวัน เพื่อศึกษาการตอบสนองต่อกลิ่นของแมลงวันทั้งสองชนิด (2) ศึกษาพฤติกรรมของแมลงวันทั้งสองชนิดต่อกลิ่นต่างๆ จากสารธรรมชาติ และสารสังเคราะห์เพื่อนำไปเป็นเหยื่อล่อที่เหมาะสม และ (3) พัฒนาสร้างเหยื่อล่อสำเร็จรูปที่เหมาะสมต่อการล่อแมลงวันทั้งสองชนิด และนำไปทดสอบในภาคสนาม วิธีการทดลองคือ ออกแบบและสร้างอุโมงค์ลมที่มีความยาว 3 เมตร แบ่งเป็น 3 ส่วนคือด้านข้างสองส่วนสำหรับวางเหยื่อ และกลุ่มควบคุมที่ไม่วางเหยื่อ ตรงกลางเป็นที่สำหรับปล่อยแมลงวัน ส่วนต่อระหว่างตรงกลางและด้านข้างทั้งสองมีกรวยสำหรับให้แมลงวันบินไปด้านหนึ่ง ด้านหลังกรวยมีอุปกรณ์สำหรับจับสัญญาณการบินเข้าของแมลงวัน สัญญาณดังกล่าวติดเชื่อมกับคอมพิวเตอร์ที่มีโปรแกรมการนับแมลงวัน ได้ทดสอบการใช้อุโมงค์ลมนี้กับแมลงวันบ้านและ *C. megacephala* และพบว่าได้ผล แต่ยังมีข้อจำกัดบางประการ จึงได้ออกแบบและสร้างอุโมงค์ลมใหม่ โดยมีความยาว 190 เซนติเมตร ความกว้าง 30 เซนติเมตร และความหนา 30 เซนติเมตร ประกอบด้วยส่วนประกอบทั้งหมด 7 ส่วนคือ (1) filter partitions 2 ส่วน ด้านนอกสุดมีพัดลมเพื่อเป็นต้นกำเนิดลม หลอดไฟเพื่อเป็นแหล่งส่องสว่าง (2) stimulus partitions 2 ส่วน เป็นที่สำหรับวางเหยื่อ (3) trapped partition 2 ส่วนเพื่อเป็นที่ดักจับแมลงวันที่บินเข้ามา และ (4) release partition 1 ส่วนตรงกลาง เพื่อสำหรับปล่อยเหยื่อเข้าไปด้านใน การทดสอบในอุโมงค์ลมนี้พบว่า เหยื่อคือเครื่องในหมูเน่า 1 วัน 300 กรัม, ความเร็วลม 0.58 m/s, เวลาการทดลองที่ 13.00-17.00 นาฬิกา, ความเข้มแสง 341.33 lux ในห้องและแสงที่ 10W สามารถดึงดูดแมลงวันได้ดีที่สุด ส่วนการทดสอบภาคสนาม ใช้เหยื่อคือเครื่องในวัวสดสำหรับดึงดูดแมลงวันบ้าน และเครื่องในวัวเน่า 1 วัน สำหรับดึงดูด *C. megacephala* สามารถดึงดูดแมลงวันให้มาที่กับดักได้เป็นจำนวนมาก

คำหลัก การควบคุม, แมลงวัน, อุโมงค์ลม, พฤติกรรมการตอบสนอง

Abstract

Project Code: RMU5080036

Project Title: Construction of dual-choice flight wind tunnel with fly number detection system for evaluation the appropriate attractive odor for house fly (*Musca domestica)* and blow Fly *(Chrysomya megacephala)* and field testing of those attractive odors as baits for both species

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Content

House fly and blow fly, *Chrysomya megacephala,* are medially important flies in Thailand. Adults are mechanical carriers of several pathogens to humans and cause annoyance, while larvae are myiasis producing agent. Regarding this, strategy to control fly population is mandatory. Three main objectives of this study included 1) design, construct and test the dual choice wind tunnel to investigate the behavioral response of both fly species, (2) investigate behavioral response of flies to several odours, both emitted from natural product and synthetic, and (3) determine the best attractive bait and evaluate its efficacy in the field. The first dual-choice wind tunnel appeared as a “T-box”, measuring 3 m, consisting of two stimulus partitions and one median release partition. The detection system consisted of the micro-controller, the detector modules, and the data logger program. Evaluation of this dual-choice wind tunnel yielded the satisfactory result for house fly and *C. megacephala*; however, still have some limitation. Thus, the new model of dual-choice wind tunnel was designed and operated. This wind tunnel consisted of seven free partitions (30×30×190 cm), (1) two “filter partitions” equipped with an adjustable fan as a wind source, a removable daylight fluorescent lamp as a light source and activated charcoal, (2) two “stimulus partitions” where that bait was placed, (3) two “trapped partition” where flies were allowed to reside, and (4) one center “release partition”. Evaluation revealed that using 1-day tainted pork viscera as baits in the wind tunnel setting 0.58 m/s wind speed at 1300-1700 h, light intensity 341.33 lux in a room and 10W as light source, yielded the highest attractive index. Field evaluation revealed that fresh beef viscera could attract house fly, while 1-day tained beef viscera could attract *C. megacephala* in high number.

Keywords: control, fly population, wind tunnel, behavioral response

4. Research content

PROBLEMS AND RESEARCH RATIONALE

House fly (*Musca domestica*) and blow fly (*Chrysomya megacephala)* are flies of medically important worldwide. In Thailand, they are extremely abundant in urban areas. Adults of both species have been proven as a mechanical transmitter of numerous disease pathogens to humans, as well as a source of annoyance. Their larvae are also myiasis producing agent. Problems arising from both species in the country result from their rapid development under warm temperatures as well as the available source of material filth as its breeding place. Regarding this, control of fly populations under the threshold of disease transmission is mandatory.

Despite the control strategy of fly populations relying typically on insecticides, some problems have been encountered. These include environmental pollution, insecticide resistance and increased pesticide costs. With the current movement towards using decreased amounts of any kind of insecticide, alternative control methods that can be included in an integrated control program should be tested and applied.

Among many control measures, the odor-baited trapping system has been employed to reduce fly population for centuries, particularly during the epidemic diseases transmitted by flies or myiasis outbreaks occurred in the economic livestock [1]. Not only for the fly control mission, the odor-baited trapping system has been used in the survey of fly population and density [2;3]. The advantages of odor-baited trapping system are non-toxic, simple and inexpensive materials under the principle of the upwind flying by the insects for locating a mate, food or a host when they have encountered a wind-borne odor released from that source [4;5]. Several natural protein sources, e.g., horse meat [2], fresh liver [5], fresh meat and pork [6], fresh animal viscera [7], putrid fish [7;8], decomposed meat [9;10] and synthetic odor source [lucilure®] [11] were employed to lure the different species of blowfly in different field studies. To develop an effective odor-bait trap method, research for the appropriate odor attracting flies has become a major priority. However, the comparative study finding the most appropriate protein odor source for medically important flies in the field is a tough work especially when analyzing the data since many external factors can not be controlled. To avoid this problem, searching for the appropriate odor source used for fly trap in the laboratory is mandatory prior to be employed in field investigation.

The wind tunnel is one of the most important tools available for studying the behavior of free-flying insects, e.g., flies [4], fruit fly [12], biting midge [13], mosquitoes [14;15], aphid parasitoid [16], beetle [17], thrips [18]. It has been used to study the nature of flight responses of insects to airborne olfactory stimuli, to observe the overall behavior of the insect and to offer many benefits in the investigation of insect behavior. There are many advantages of the wind

tunnel over other methods, such as the odor gradient created along the line of the air flow in the wind tunnel is closed to the odor formed by a distant source in nature. By this odor, the insect can use the positive anemotaxis for navigation toward the odor source in the same way that it might in the field [19]. In addition, the use of a wind tunnel over field studies in the initial stages of olfactory attractant identification include greater control of the variables that modulate response, higher discriminating power, and the ability to conduct experiments throughout the year or during inclement weather [20]. The positive responses in wind tunnels provide the most convincing evidence that an odor source is truly attractive to an insect [21].

Concerning such published information, it was the objective of this study to designed, constructed and operated the new wind tunnel model to improve the efficacy of wind tunnel for evaluating behavioral response of fly. Such improved model was employed to comparatively evaluate the efficacy of flies to 13 kinds of bait under laboratory conditions. Moreover, the present study was designed to assess the attractive index of flies while fasting, to determine the active time for feeding, to determine the appropriate weight of bait and to evaluate the suitable wind speed and light intensity operated in the wind tunnel. This allowed the most appropriated conditions used to assess behavioral response of flies in the wind tunnel.

OBJECTIVES

1. To design, construct and operate the new wind tunnel model to improve the efficacy of wind tunnel for evaluating behavioral response of fly
2. To investigate behavioral response of flies to several odours, both emitted from natural product and synthetic
3. To determine the best attractive bait and evaluate its efficacy in the field.

MATERIALS, METHOD AND RESULTS

Rearing house fly and blow fly *C. megacephala* in the laboratory

Adult flies were maintained under ambient temperature and natural conditions in the fly rearing room at the Department of Parasitology, Faculty of Medicine, Chiang Mai University. Adults were reared in rearing cages (30×30×30 cm) screened with black cloth. Adults were reared on two kinds of food; (I) a mixture of 10% (w/v) sucrose solution at 985 ml and multivitamin syrup (Syn-O-Vits: Thailand) at 15 ml; and (II) fresh pork liver as both a food source (protein) and oviposition site. Small pieces of 40 g fresh pork liver was placed in a glass petri dish (9 cm in diameter) and changed daily. The dish with the liver was located at the bottom of the cage. A plastic cup (5×4 cm), with a hole centrally located in the lid, was used to contain the mixture of sucrose solution and multivitamin syrup. A wick (10 cm in length) was

inserted through the hole in the lid and used as a feeding site for the adult flies, and it was changed on alternate days. Subsequently, the oviposition sites were observed daily for the presence of fly eggs; and if present, they were transferred to a 12×15×6 cm transparent plastic box, with 40 g of fresh pork liver provided as larval food. The lid of the box had a rectangular-shaped hole cut into 3/4 of its total area, which was covered with the finest silk screen cloth (100 meshes/mm^2) for ventilation as well as preventing other small insects from entering the rearing box to oviposite. The box was covered by the lid and sealed tightly with adhesive paper tape to prevent the larvae from crawling out. It was kept under room temperature (24-28 °C) in a cabinet at the rearing room of the Department of Parasitology. Liver was replaced daily until some third instars developed into prepupa, the nonfeeding period. The box with pupae was still covered and tightly sealed until some pupae emerge as adults. Then, it was placed into a rearing cage before the lid was taken off to release the adults into the cage.

Design, construct and operate wind tunnel model for evaluating behavioral response of fly

The dual-choice wind tunnel was constructed from acrylic coated plywood supported by a stainless steel frame. This wind tunnel was 300 cm in length and equally divided into 3 partitions, one release partition at the middle and two stimulus partitions at both sides (Figs. 1, 2). The cross-section dimensions of each partition were 60 cm by 60 cm. Another trapezoidal box (100 cm in length and 60 cm in height) was attached to the middle part of wind tunnel to increase the release area. A removable nylon fly net was installed to fit inside each partition for gathering and evacuating the flies after experiments. Each stimulus partition was illuminated by a fluorescent daylight tube (10W) (Nulite®, Thailand). Room air was blown through the activated charcoal filter (HAC-2®, Thailand) into the wind tunnel by alternating current electric ventilating fans (Scaner® ST-60, Thailand) installed at both ends of stimulated partitions, one fan per one partition. The motor speeds of these fans were controlled by a loop-back speed controller. One humidifier was placed in the space between the filter and the ventilating fan in each stimulated partition. Two 3 mm thick white opaque polypropylene plates were installed to separate the releasing partition from the stimulating partitions. Four trapping funnels were placed in 2 rows with a 5 cm gap between each module in the central portion of each polypropylene plate. Each entrance consisted of 2 parts; the white plastic conical opening portion and the entrance module. The diameter of opening portion was 10 cm while the opaque white acrylic entrance module was divided into 3 accesses of 0.9 cm in width, 1.0 cm in height, and 3.0 cm in depth.

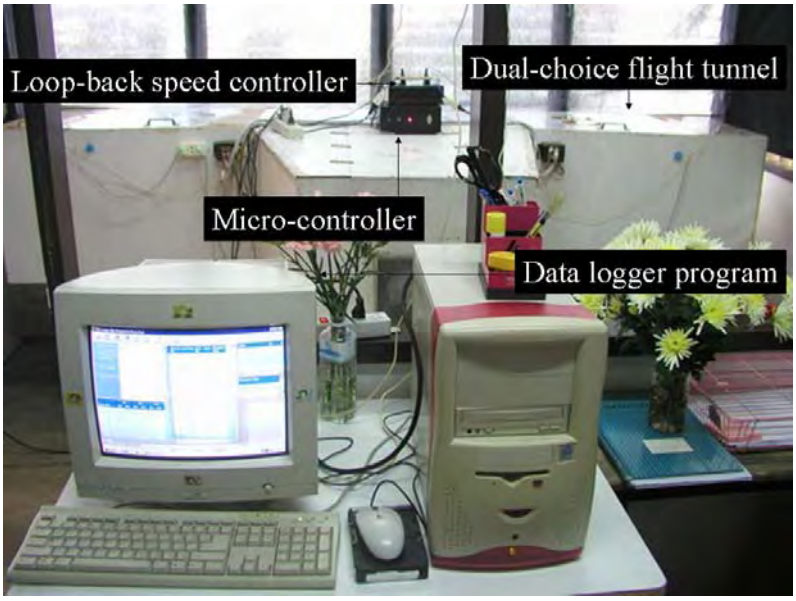


Fig 1. Photograph displaying all equipments that were designed and constructed in this study, comprising the dual-choice flight tunnel, micro-controller, data logger program, loop-back motor speed controller.

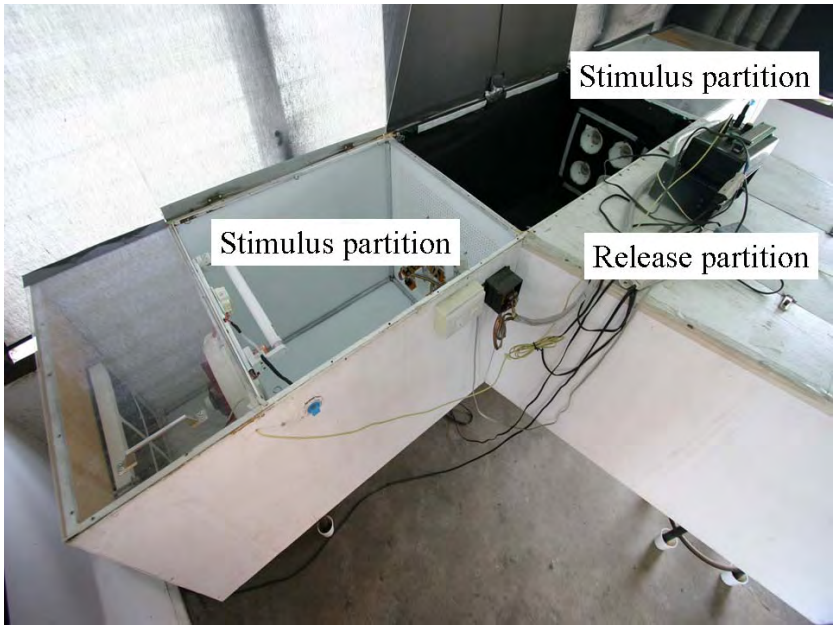


Fig 2. Top view of photograph displaying inside of dual-choice flight tunnel, composing laterally two stimulus partitions and one medial release partition.

Detection system

The detection system consisted of the micro-controller, the detector modules, and the data logger program.

Micro-controller

The micro-controller was a coordinator that controlled coordinately between the detector modules and the data logger program. It was programmed for receiving the data from the detector modules, screening and selecting only the positive output that determining the interruption of infrared beam, and lastly transferring the screened information to the data logger program for further analysis of the results. This component was controlled by the data logger program. It was connected to each detector module and the data logger program via a 10-core ribbon cable and a phone wire, respectively. It started to operate whenever it was activated by the signals from the data logger program. The photograph of the electronic board of the micro-controller was demonstrated in Fig 3.

Fig 3. Photograph showing the interior of the micro-controller.

Detector modules

The detection system was consisted of 24 sets of detector modules (Fig. 4); 12 sets per side of the tunnel. The accuracy of each detector module set was assessed by introducing the dead fly individually into the gate (represented as the fly was entering into the partition) and

taking them out from the gate (represented as the fly was going out from the partition). The body sizes (mean±SD) of the dead male and female flies used in this experiment were 3.53±0.15 mm in width and 9.25±0.46 mm in length.

The accuracy of each detector set is varied, ranging from 80.00% to 96.67%. No statistical different was found between the mean accuracy of the detector modules which detected as “in” or “out” (t-test, t = 0.252, df = 46, P = 0.802), suggesting that the directions of recognition did not affect the accuracy of the detector modules. Comparing the accuracy of detector modules in each side of the tunnel, the mean accuracy of the detector modules for right side (Gate 1-4) and left side (Gate 5-8) were 90.41±3.72% and 88.33±3.54%, respectively. However, no significant difference between the accuracy of detector modules for the right and left sides was observed (t-test, t = 1.988, df = 46, P = 0.053). The absolute accuracy of detector modules of this detection system was 89.38 ± 3.74%.

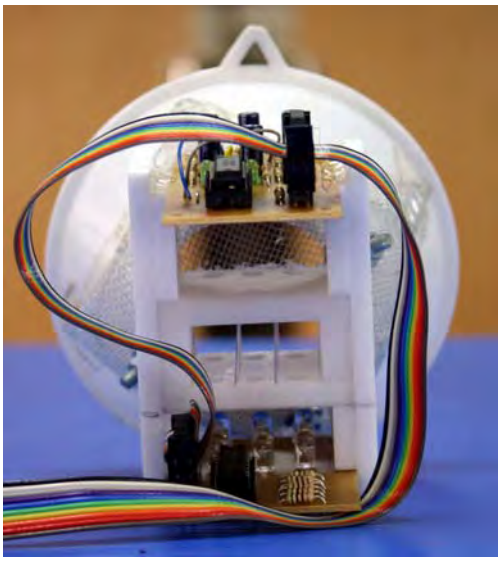
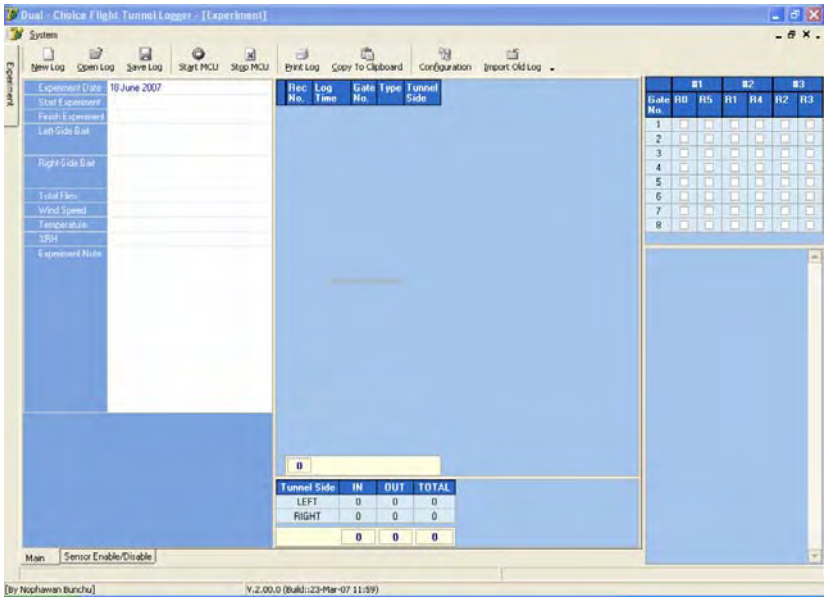


Fig 4. Photograph of the fly entrance showing the back view adhering with the detector modules.

Data logger program

The data logger program for operating with the detection system was named as “FT-Logger or Dual-choice Flight Tunnel Logger Program”. The main functions of this program were to translate the digital signals from the micro-controller to be the results, to collect the data for analysis, and to display the results on the computer. It was programmed to process only the defined signals that were sent from the first and the second infrared modules within 60 sec,

indicating that it translated only a pair of the digital signals, sent from the first and the second modules within 60 sec. If the second signal is delayed from the first signal over 60 sec, the first signal will be cleared and the data logger program is not process that signal. This program was operated on Microsoft Windows 98/ME/XP/2000/2003. An example view of the data logger program operating on the computer was shown in Fig 5.



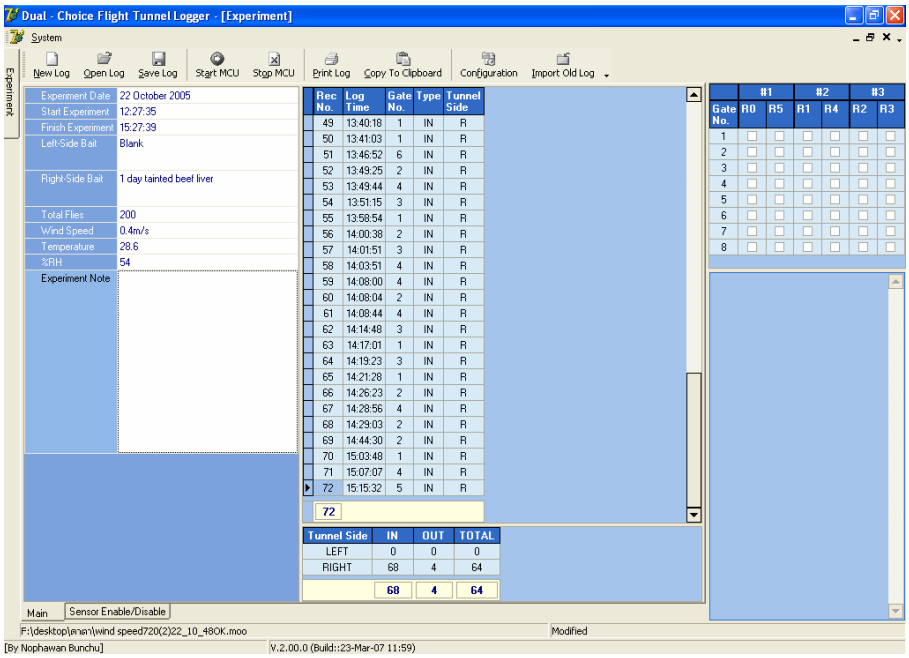


Fig 5. An example view of the dual-choice flight tunnel logger for recording the number of responding flies in the dual-choice flight tunnel (after the experiment has finished).

Operation of the detection system

All of the components of this system worked coordinately. This system started to operate whenever all of components were activated. Firstly, the data logger was opened and then it sent the digital signal to the micro-controller to activate its function. Secondly, the micro-controller sent the electric signals to each detector module to activate the infrared emitter diode and the infrared receiver diode, which are the main components of the detector module. The infrared-emitting diode was set to emit the infrared wave as frequency 38 KHz regularly, because the receiver diode was specific to only that frequency. All detector modules sent repeatedly the digital signals to the micro-controller. Thereafter, the micro-controller was programmed to process the signals and select only the signal that determined the interruption of infrared beam for sending the data back to the data logger program later. Finally, the program translated each digital signal from the micro-controller to be an individual result of each responding fly including a record number, a log time of response, a number of gate that fly enter or escape, a type of response (“in” or “out” the stimulus partition), and a side of the stimulus partition (right or left partition).

Efficiency of the detection system

The accuracy of the detector modules for detecting number of flies that entered into or escape from the partition was ≈90% when it was tested with the dead flies. Unfortunately, when the detection system dealt with the alive flies, its efficiency was decreased. The accuracy of the detection system was evaluated from the difference between manual and detector counts of the total sixty experimental data. The mean difference between the manual and detector counts differed significantly (Wilcoxon Signed Rank test, Signed Rank = -5.012, P=0.00). The error of this detection system varied, ranging from -66.67% to 77.78%. The mean absolute value of error (±SE) was 39.09±24.18%. On the other hand, the accuracy of this detection system was 58.65± 19.99%, ranging from 22.22% to 100%. Although the manual (m) and detector (d) counts were not equal, the regression analysis revealed a significant linear relationship between them, with the regression line being m = (11.7663±6.1746) + (1.4892±0.0997)d (F=223.200, P<0.0001, R^2=0.793, Adjusted R^2=0.790). The accuracy of the detection system was decreased ≈34.35%, when compared with the absolute accuracy of the detector module.

Efficacy comparison of the wind speed on the behavioral response of flies to olfactory stimuli

Fig 6 displayed the calibration curve of the wind speed inside the dual-choice flight tunnel. The equation of this curve yielded y = 0.0007x - (0.1177±0.0460), (F= 186.8979, P=0.0008,

R^2=0.984, Adjusted R^2=0.979) where x was motor speed (r.p.m.) and y was wind speed (m/s). From this equation, the wind speed operated at 0.1, 0.2, 0.3, 0.4, and 0.5 m/s expressed 292, 435, 577, 720, and 863 r.p.m. of the motor speed, respectively. These motor speeds were set for studying in this experiment. During these experiments, the mean temperature (±SD) and mean relative humidity (±SD) measured 28.07±2.00 °C and 64.30±9.19%, respectively.

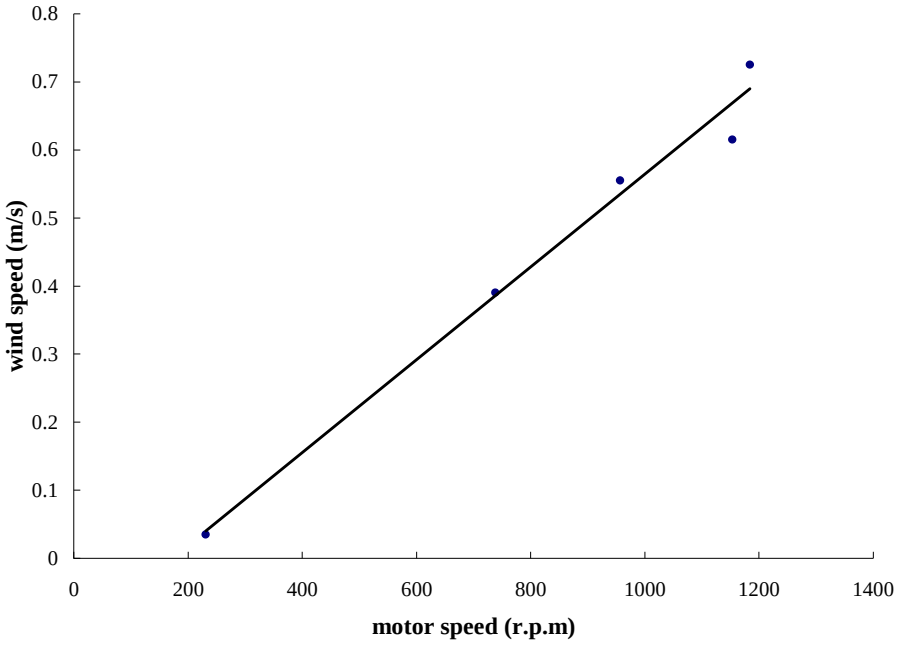


Fig 6. Calibration curve of wind speed inside the dual choice flight tunnel;
y = 0.0007x - (0.1177±0.0460), R^2 = 0.984.

The comparison of the attractiveness of the natural products was assessed based on the manual count. In general, no significant difference was detected in males and females responded to all wind speed levels (Pearson Chi-Square = 0.282, *df* =1, *P*=0.595), thereby suggesting the data to be pooled for analysis. Figure 24 showed the percent of responding flies that entered in each partition at each wind speed level; whereas, the positive correlation between the wind speed and percent of responding flies was observed (Fig. 7). The regression line yielded y = (43.6667±8.0254) + 200.00±26.5070x (*F*= 56.9299, *P*=0.0017, R^2=0.934, Adjusted R^2=0.918). The results indicated that at different wind speed level, flies responded to odor significantly different (Pearson Chi-Square = 365.895, *df* = 10, *P*=0.000). At wind speed operated at 0 m/s, most of the flies (54.5%) entered into the partition that was contained nothing; whereas, there were only 20% and 25.5% of the tested flies in the odor-loaded and the

middle partitions, respectively. In contrast, the number of responding flies entering into the odor-loaded partition increased when the wind speed was set at higher level. Simultaneously, the number of flies that entered into the blank and the middle partitions was therefore decreased. In the present study, the highest number of responding flies (≈70%) entering into the odor-loaded partition was observed when the wind speed was set at 0.5 m/s. In this regard, the wind speed level set at 0.5m/s was selected to be used in the subsequent experiments.

Although the total number of responding males and females was not significant difference, the responding males and females were found significantly different in some wind speed levels. For instance, at wind speed set at 0.1 and 0.2 m/s, more number of responding males was observed than females (Pearson Chi-Square = 3.954, *df* =1, *P*= 0.047 and Pearson Chi-Square = 6.285, *df* =1, *P* =0.012, respectively). In contrast, more number of responding females was observed significantly higher than males (*Pearson Chi-Square* = 12.734, *df* =1, *p* =0.000) when the wind speed set to be 0.3 m/s.

Analysis of the data from the data logger program revealed that flies responded to each wind speed level with a different velocity. At the wind speed set to be 0.5 m/s, the first fly took <60 sec to reach the bait and the 1-day tainted beef liver.

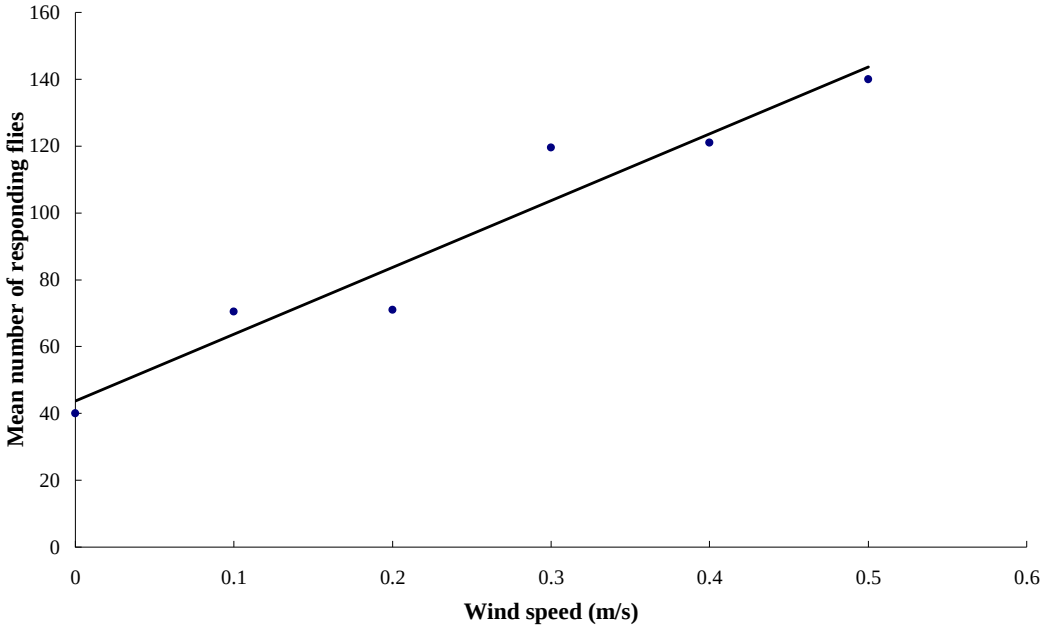


Fig 7. Positive correlation between the wind speed set in this experiment and the mean number of responding flies; y = (43.6667±8.0254) + 200.00±26.5070x, R^2=0.934.

Behavioral response of *C. megacephala* to odor emitted from natural products: Screening odors in the rearing cage

Seventy-two kinds of natural products that have strong odors were selected to assess the behavioral response of flies in the rearing cage. The fresh natural products were purchased from local retailers and either used immediately or kept in a plastic bag left in ambient condition for 1 or 3 days to make them tainted. The assessment was performed in the rearing cages (30×30×30 cm) between 12.00 P.M. and 3.00 P.M. at the ambient temperature of the laboratory using the method of Chaudhury et al. [22] with some modifications.

The tested natural products were divided into 4 categories: (1) fresh animal-origin materials, (2) fresh plant-origin materials, (3) preserved food, and (4) 1- and 3-day tainted animal- or plant-origin materials. One hundred adult males or females were placed in a cleaned rearing cage. All were aged 5-7 days old and deprived of food and water 24 hours prior to each trial. After 5 minutes to acclimatize, 200 gm of a natural product placed on a clean glass plate (Ø=15 cm) was introduced to the cage. Flies were considered to respond to the natural product when they had landed and/or stayed still on the natural product within 5 minutes. Only the responding flies were counted and recorded. The trial of each natural product was repeated 3 times with different groups of flies. At the end of each trial, the cages were cleaned by rinsing with hot water and the new fabric sleeves were replaced. The natural products that attracted >50% of the total flies were used as the candidate odor sources for further study in the dual-choice wind tunnel in the subsequent experiment.

Behavioral response of *C. megacephala* to odor emitted from natural products: Response in the dual-choice wind tunnel

The behavioral response of *C. megacephala* in the dual-choice wind tunnel was investigated using the procedures previously described. To control for any possible side bias, the position of the tested natural product and standard bait were changed in every experiment. In addition to the number of responding flies in each partition, the percentage of the trapped flies of each sex was calculated to analyze the behavior of each sex toward each natural product odor. The potency of the attractiveness of each natural product was determined following the formula described by Urech et al. [23] as

$$\text{Potency of attraction for natural product (X)} = \frac{\text{Mean no. of flies attracted to X}}{\text{Mean no. of flies attracted to standard bait}}$$

where the 1-day tainted beef liver was the standard bait.

A total 27 kinds of natural products (9 fresh and 18 tainted animal-origin materials) yielded the attraction >50% (Tables 1-5). Among these, only fresh pork liver and some kinds of the 1-day or 3-day tainted animal products (beef viscera, pork viscera and beef) attracted $\geq$ 90% of the tested flies in both sexes. The average percentages of responding flies toward odors of natural products are shown in Table 1. The most attractive material for *C. megacephala* was 1-day tainted pork viscera, with the percentage of attraction of both sexes being ≈95%. In contrast, the plant-origin materials and preserved foods exhibited less attraction to lure *C. megacephala*. None of the plant-origin materials nor the preserved food materials could attract >50% of the tested flies. Ripe jackfruit displayed the best attraction among plant-origin materials, indicating ≈40% of the attractiveness for both sexes. Regarding preserved food material, sardines in tomato sauce revealed the best attraction, but with attractiveness ≈32% in both sexes.

Table 1. Average of responded *Chrysomya megacephala* to odors emitted from animal-origin materials.

Natural products	% Responding flies	
	Male (Average)	Female (Average)
Pork liver *,[c]	90.67	90.00
Chicken intestine (Female) *,[a]	82.33	88.67
Pork bone *,[a]	80.00	94.00
Beef *,[c]	70.87	72.33
Chicken bone *,[c]	66.33	70.00
Beef viscera *,[c]	64.33	69.50
Pork viscera *,[a]	60.33	52.00
Chicken viscera *,[c]	58.33	53.33
Beef liver [c]	57.67	58.00
Duck liver [c]	49.33	49.33
Duck viscera [c]	49.33	45.67
Duck heart [c]	45.33	48.00
Swine blood [b]	41.00	21.67
Minced pork [a]	38.67	52.67
Stream Indian mackerel [c]	33.33	35.00
Bovine blood [c]	32.67	26.67
White vanamike shrimp [c]	25.67	23.00
Batrachian walking cat fish [c]	21.00	25.33
Chicken eggs [c]	2.67	2.00
Fish viscera of *Oreochromis niloticus* [c]	1.67	1.33

* The natural product that could attract flies >50% in 5-min period.
[a] Females had a significantly higher mean number of flies than males (P<0.05).
[b] Males had a significantly higher mean number of flies than females (P<0.05).
[c] Mean number of males were not statistically different from females (P>0.05).

Table 2. Average of responded *Chrysomya megacephala* to odors emitted from plant-origin materials.

Natural products	% Responding flies	
	Male (Average)	Female (Average)
Ripe jackfruit [b]	48.33	35.67
Ripe sugar apple [b]	42.30	32.13
Ripe banana [c]	38.66	46.22
Ripe banana 2 [c]	34.68	34.33
Fresh Chaom [c]	34.33	34.33
Coconut [c]	33.67	32.67
Ripe breadfruit [c]	29.67	35.33
Ripe Indian mulberry [c]	22.89	22.00
Boiled Chaom [c]	20.67	20.00
Cooked Jasmine rice [c]	18.33	16.00

[b] Males had a significantly higher mean number of flies than females ($P<0.05$).
[c] Mean number of males were not statistically different from females ($P>0.05$).

Table 3. Average of responded *Chrysomya megacephala* to odors emitted from preserved food.

Natural products	% Responding flies*	
	Male (Average)	Female (Average)
Sardine in tomato sauce (Roza®, Thailand)	31.67	34.33
Dog food (Minced liver and meat, OK®, Thailand)	25.74	31.67
Tuna steak in oil (Nautilus®, Thailand)	23.67	22.67
Dried spanish mackerel	12.55	17.74
Fermented soy bean	8.00	13.67
Powder milk (S-26 Promil Gold®, Singapore)	7.00	9.33
Fish sauce (Tesco ®, Thailand)	6.67	9.00
Salty fish	4.00	5.33
Shrimp paste	2.33	2.33
Fermented fish	2.33	2.33

* Mean number of males were not statistically different from females ($P>0.05$).

Table 4. Average of responded *Chrysomya megacephala* to odors emitted from tainted materials.

Natural products	% Responding flies	
	Male (Average)	Female (Average)
1-day tainted pork viscera *, c	94.33	95.67
3-day tainted pork viscera *, c	93.33	94.67
3-day tainted beef *, c	92.33	93.33
1-day tainted beef viscera *, c	91.33	91.67
1-day tainted beef *, c	89.33	90.00
3-day tainted beef viscera *, c	88.67	91.67
1-day tainted Batrachian walking Catfish *, c	85.67	85.67
1-day tainted chicken intestine *, c	82.67	86.00
1-day tainted pork bone *, a	80.00	86.67
1-day tainted swine blood *, c	78.00	75.00
1-day tainted pork *, c	77.00	81.33
3-day tainted bovine blood *, b	70.33	51.67
1-day tainted pork liver *, c	69.33	69.33
3-day tainted chicken bone *, c	68.00	74.33
1-day tainted chicken bone *, c	64.33	70.33
3-day tainted pork liver *, a	57.33	77.00
1-day tainted chicken viscera *, c	52.25	56.67
1-day tainted beef liver *, c	50.67	55.67
1-day tainted bovine blood c	44.00	43.67
3-day tainted beef liver c	41.33	45.00

Table 4 (Continued).

Natural products	% Responding flies	
	Male (Average)	Female (Average)
3-day tainted soup bone [c]	39.33	41.00
1-day tainted duck viscera [c]	38.67	45.67
3-day tainted swine blood [c]	37.33	35.33
3-day tainted chicken intestine [c]	36.00	53.33
3-day tainted chicken eggs [b]	33.67	18.33
3-day tainted chicken viscera [c]	32.00	33.00
3-day tainted Batrachian walking [c] Catfish [a]	31.33	41.00
3-day tainted coconut [c]	29.00	27.33
3-day tainted pork [a]	26.33	49.33
3-day tainted duck viscera [c]	24.00	28.00
1-day tainted chicken eggs [c]	2.67	1.33
1-day tainted coconut [c]	2.00	1.67

* The natural product that could attract flies >50% in 5-min period.
[a] Females had a significantly higher mean number of flies than males (P<0.05).
[b] Males had a significantly higher mean number of flies than females (P<0.05).
[c] Mean number of males were not statistically different from females (P>0.05).

Table 5. The natural products that could attract more females than males *Chrysomya megacephala*.

Natural products	*P* value	Pearson Chi-Square
3-day tainted pork	0.000	33.738
3-day tainted pork liver	0.000	26.308
Soup pork bone	0.000	25.995
3-day tainted chicken intestine	0.000	18.234
Pork	0.001	11.849
3-day tainted Catfish	0.014	6.071
Fermented soy bean	0.017	5.667
Chicken intestine	0.028	4.853
1-day tainted soup bone	0.028	4.800
Pork viscera	0.040	4.231

Table 6. The natural products that could attract >50% of *Chrysomya megacephala* in the screening cage.

Natural products	% Responding flies	
	Male (Average)	Female (Average)
1-day tainted pork viscera	94.33	95.67
3-day tainted pork viscera	93.33	94.67
3-day tainted beef	92.33	93.33
1-day tainted beef viscera	91.33	91.67
Fresh pork liver	90.67	90.00
1-day tainted beef	89.33	90.00
3-day tainted beef viscera	88.67	91.67
1-day tainted Batrachian walking catfish	85.67	85.67
1-day tainted chicken intestine	82.67	86.00
Chicken intestine (Female)	82.33	88.67
1-day tainted soup bone	80.00	86.67
Soup pork bone	80.00	94.00
1-day tainted swine blood	78.00	75.00
1-day tainted pork	77.00	81.33
Fresh beef	70.87	72.33
3-day tainted bovine blood	70.33	51.67
1-day tainted pork liver	69.33	69.33
3-day tainted chicken bone	68.00	74.33
Fresh chicken bone	66.33	70.00
1-day tainted chicken bone	64.33	70.33
Fresh beef viscera	64.33	69.50
Fresh pork viscera	60.33	52.00
Fresh chicken viscera	58.33	53.33
Fresh beef liver	57.67	58.00
3-day tainted pork liver	57.33	77.00
1-day tainted chicken viscera	52.25	56.67
1-day tainted beef liver	50.67	55.67

In regard to the behavioral response of flies within the screening cage *per se*, the first fly attracted to the odor generally landed on the material within 2-3 seconds after the natural product was placed in the cage. Most flies that landed on the tested materials protruded their proboscises to lap the material and walked around the plate. However, some flies ignored the odor by resting on the wall of the cage or walked around the floor. Interestingly, the natural products that emitted strong odors recognized by humans (e.g., salty fish, shrimp paste, fermented fish) could not attract flies to land on them, but flies responded strongly to those odors by orienting actively over those materials.

In general, no significant difference was observed between males and females in responding to most of the natural products. However, some natural products were more attractive effect to one particular sex. The 3-day tainted pork, 3-day tainted pork liver, pork bone, 3-day tainted catfish, fermented soy bean, chicken intestine and ovary, 1-day tainted pork bone and pork viscera attracted more females than males (P<0.05). In contrast, the swine blood, 3-day tainted bovine blood, 3-day tainted chicken eggs, ripe jackfruit and ripe sugar apple attracted more males than females (P<0.05).

Behavioral response of *C. megacephala* to odor emitted from natural products: Response in the dual-choice wind tunnel

In all trials of the 24 kinds of natural products tested, significant difference among number of flies in the odor-loaded, odor-free, and middle partition was detected (P<0.05). The 1-day tainted pork viscera attracted the highest number of responding flies, 90.75% of the tested flies. On the other hand, the fresh chicken bone was the least attractive material in these trials, attracting 56.75% of the responding flies.

In terms of the potency of attractiveness, the 1-day tainted pork viscera was the most attractive to *C. megacephala*, having a potency of 1.578 (Table 7). In contrast, only three kinds of the natural products (i.e., fresh chicken bone, 1-day tainted swine blood and fresh beef) had a lower potency (potency = 0.987-0.996) than the standard material (1-day-tainted beef liver).

In the experiment conducted in the dual-choice wind tunnel, the averages (mean±SD) of the temperature and relative humidity were measured 27.30±3.20°C and 63.09±7.85%, respectively. Flies responded continually to the tested materials during the 3-hour experimental period. The first fly generally entered into the odor-loaded partition within 2 minutes after the natural product was introduced into that partition, and the last fly that entered into that partition was still found up to the last minute of the experiment. Although they did not enter into the odor-loaded partition, most flies remained in the middle partition, preferring to rest on the area or fly

nearby the odor-loaded partition. To confirm this observation, the natural product was moved to another partition; flies also changed the resting position to the other side.

Table 7. Potency of the attractiveness of *Chrysomya megacephala* toward natural products.

Natural product	Potency*
1-day tainted pork viscera	1.578
Fresh pork bone	1.483
1-day tainted chicken bone	1.474
1-day tainted beef viscera	1.387
3-day tainted chicken bone	1.357
Fresh beef viscera	1.335
1-day tainted chicken viscera	1.322
1-day tainted cat fish	1.313
3-day tainted beef viscera	1.309
1-day tainted beef	1.300
1-day tainted pork bone	1.283
3-day tainted pork viscera	1.104
3-day tainted beef	1.104
Fresh chicken viscera	1.083
3-day tainted bovine blood	1.052
1-day tainted pork liver	1.043
3-day tainted pork liver	1.043
Fresh pork liver	1.026
Fresh pork viscera	1.013
Fresh beef liver	1.013
Fresh beef	0.996
1-day tainted swine blood	0.991
Fresh chicken bones	0.987

* Potency of natural product (X) = Mean number of flies attract to X/mean number of flies attract to 1-day tainted beef liver.

Behavioral response of house fly to odor emitted from natural products: Screening odors in the rearing cage

Forty-two kinds of natural products that have strong odors were selected to assess the behavioral response of house fly in the rearing cage. The fresh natural products were purchased from local retailers and used immediately. The method of assessment was performed as previously described for *C. megacephala*. The average of responsed of *Musca domestica* to odors emitted from animal-origin and plant-origin materials were shown in Tabls 8 and 9, respectively.

Table 8. Average of responded *Musca domestica* to odors emitted from animal-origin materials

Natural products	% Responding flies	
	Male (Average)	Female (Average)
Beef viscera *[b]	71.33	76.67
Pork liver *,[a]	63.00	74.33
Bovine blood	59.67	64.00
Chicken intestine (Female) *,[a]	55.67	67.67
Minced pork [a]	54.00	63.00
Beef liver [b]	59.00	57.67
Batrachian walking cat fish [a]	28.00	36.67
Pork viscera *[a]	22.33	42.00
Swine blood [a]	17.00	33.00
Stream Indian mackerel [a]	9.67	15.67

* The natural product that could attract flies >50% in 5-min period.
[a] Females had a significantly higher mean number of flies than males (P<0.05).
[b] Males had a significantly higher mean number of flies than females (P<0.05).

Table 9. Average of responded *Musca domestica* to odors emitted from plant-origin materials

Natural products	% Responding flies	
	Male (Average)	Female (Average)
Ripe banana	64.00	59.00
Coconut [b]	64.00	53.67
Cooked Jasmine rice	53.33	61.67
Molasses	40.00	39.00
Sugar [a]	15.33	24.00

[a] Females had a significantly higher mean number of flies than males (P<0.05).

[b] Males had a significantly higher mean number of flies than females (P<0.05).

Design, construct and operate NEW wind tunnel model to improve the efficacy of wind tunnel for evaluating behavioral response of fly

The dual-choice wind tunnel consisted of seven free partitions (30×30×190 cm) (Fig. 8). The ends of tunnel consisted of “filter partitions” made from wood, each equipped inside with an adjustable fan (12V DC, ∅=30 cm) as a wind source, a removable 10 or 100W daylight fluorescent lamp as a light source and 1 kg activated charcoal on a plastic tray and a filter sheet as an air cleaner. To adjust the wind speed at a release partition, fans were switched the voltage to 0V, 4.5V, 6.0V, 7.5V, 9V and 12.0V, producing an average wind speed of 0.13, 0.58, 0.72, 0.85, 0.93 and 1.08 m/s, respectively, and the direction of air flow was move centrally (Fig. 8, arrows). The “stimulus partitions” made of 4 transparent sheet glasses with aluminium frame (30×30×30 cm) were the attractant parts where the bait was placed in one side. The next two interior partitions “trapped partition”, made of 4 transparent sheet glasses with aluminium frame (30×50×30 cm), where flies were allowed to reside. The black net bags were placed within this part for collecting flies. The center one was the “release partition” made of 4 transparent sheet glasses with aluminium frame (30×30×30 cm). The upper part of release partition was a center-holed transparent sheet glass (∅=6 cm) for releasing flies. The front and the top floor parts were made from 2 transparent sheet glasses, thus allowing observe fly movement interiorly; whereas two lateral parts was the slide door of the transparent sheet glasses for releasing flies into the trapped partition.

Prior to each experiment, all glass partitions were well washed to eliminate the odour with commercial cleaning solution and allowed to naturally dry at least 24 h before experiments. Two wooden filter partitions were wiped up using acetone (grade) on a sponge and allowed to dry at least 24 h.

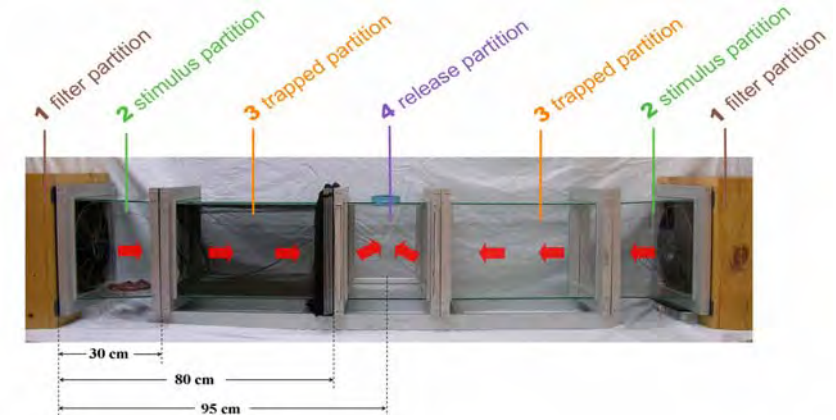


Fig 8. Dual-choice wind tunnel seven free partitions (30×30×190 cm). Two “filter partitions”, each equipped inside with an adjustable fan (12V DC, ∅=30 cm) as a wind source, a removable 10 or 100W daylight fluorescent lamp as a light source. Two “stimulus partitions” for odour release from bait. Two “trapped partitions” and one centrally “release partition” for releasing flies.

Adult *C. megacephala* was evaluated their responses within the wind tunnel in air-temperature controlled room (25-30°C) with light intensity of 341.33 lux and ambient humidity during 1300-1700 h. Sixty well-fed flies (30 females, 30 males) were transferred into a release partition and left to acclimatize for 30 min. Then, 300 g of bait placing on a glass dish (∅=14 cm) was put at one side of the stimulus partition, while the other was empty. The fans equipped at the filter partitions were set at 4.5 V, producing wind speed of 0.58 m/s, while the light sources were provided with 10W daylight fluorescence lamps. The lights and fans were turned on to produce illumination and push the air (and odour) throughout the wind tunnel steadily, respectively, for 30 min. Next, the side doors of the release partition were slid outward to allow flies moving into each trapped partition for 1 h. At the end of the experiments, the net bags with responded flies were removed. Flies were sacrificed by placing the net bags in the freezer (-18 ºC) for 10 min and then counted. The attractive index, based on Stensmyr et al. [24] was calculated from

$$\frac{\text{No. flies in odour -trapped partition} - \text{No. flies in odour free-trapped partition}}{\text{Total flies } (n = 60)}$$

Assessment kinds of bait

Thirteen products from 5 kinds of baits (i.e., pork viscera, pork liver, beef viscera, beef liver and mackerel) were evaluated for their efficacy to attract *C. megacephala* in the wind tunnel. Each kind was categorized into fresh (or kept in freezer set at -18°C less than 2 days), 1-day tainted and 3-day tainted bait. The 5 fresh products were purchased from a local market. Pork viscera consisted of 4 kinds of organs — meat, heart, intestine and liver in equal; while beef viscera consisted of 3 kinds — meat, crop and liver in equal. These products were chopped into small pieces (5×5×1 cm/pieces). For tainted baits, the chopped products were placed in an average 37 °C (35-38 °C) incubator (Siam®, Thailand) with 40-68 %RH for 24 h (1-day tainted) or 96 h (3-day tainted). These kinds of bait were utilized in the same manner as the wind tunnel experiment, with the wind speed set at 0.58 m/s. All experiments were performed 3 replications.

The study was conducted to assess the fasting of flies against three kinds of bait— (1) 1-day tainted pork viscera, which was the type of high attractive index, (2) fresh beef liver and 3-days tainted beef viscera, which were the types of low attractive index. Before starting the experiments, flies were ensured fasting conditions by providing only water for 24 h, whereas granulated sucrose as the regular carbohydrate source was omitted. The experiment was performed in the same manner as the wind tunnel described above. All experiments were performed 3 replications.

Using 1-day tainted pork viscera and 1-day tainted beef viscera as baits in the wind tunnel setting 0.58 m/s at 1300-1700 h yielded the highest attractive index of *C. megacephala*, with the median 0.63 and 0.60, respectively ($p<0.05$) (Table 10). Using mackerel as baits has proved that this kind was the least attractive for adult flies, either fresh, 1-day tainted or 3-day tainted.

Table 10. Attractive index of *Chrysomya megacephala* responded to bait in the wind tunnel

Flies	*n*	Bait (300 g)	Attractive index*
well-fed	180	1-day tainted pork viscera	0.63(0.50 to 0.65)^{a}
	180	1-day tainted beef viscera	0.60(0.47 to 0.62)^{a}
	180	1-day tainted pork liver	0.40(0.25 to 0.50)^{ab}
	180	Fresh pork viscera	0.28(0.17 to 0.30)^{b}
	180	3-day tainted pork viscera	0.28(0.20 to 0.33)^{b}
	180	1-day tainted beef liver	0.23(0.20 to 0.25)^{bc}
	180	Fresh pork liver	0.22(0.20 to 0.30)^{bc}
	180	Fresh beef viscera	0.17(0.13 to 0.30)^{bc}
	180	3-day tainted beef viscera	0.10(0.08 to 0.37)^{bc}
	180	Fresh beef liver	0.05(0.02 to 0.22)^{c}
	180	Fresh mackerel	0.02(0.00 to 0.03)^{c}
	180	1-day tainted mackerel	0.02(0.00 to 0.03)^{c}
	180	3-day tainted mackerel	0.00(-0.03 to 0.02)^{c}
24-h starved	180	1-day tainted pork viscera	0.45(0.23 to 0.50)^{ab}
	180	Fresh beef liver	0.18(0.05 to 0.20)^{bc}
	180	3-day tainted beef viscera	0.13(0.08 to 0.22)^{bc}

Wind speed was set at 0.58 m/s in the laboratory experiment at 25.0-30.0˚C, 36-77% relative humidity.

*Data are expressed as median (range) of combined females and males in three independent experiments, and different letters deemed significantly (p<0.05).

Assessment fly active time and weight of 1-day tainted pork viscera

The assessment of fly active time was performed in the same manner as the wind tunnel experiment, but the duration time was focused on 0900-1200 h and 1300-1700 h. One-day tainted pork viscera (100 g, 300 g, and 1 kg) was utilized for these experiments. The experiments were carried out triplicates.

The high attractive index of 0.60 or 0.63 suggested that the duration of the experiment at 1300-1700 h was the most active time of flies in the wind tunnel (Table 11). The wind speed at 0.13 m/s and 0.58 m/s yielded no significant difference in high attractive index. As for the weight of 1-day tainted pork viscera was concerned, using 100 g and 300 g 1-day tainted pork viscera caused no difference in attractive index in both wind speed. However, using 1 kg of 1-day tainted pork viscera resulted in low attractive index in wind speed set at 0.13 m/s.

Table 11. Attractive index of *Chrysomya megacephala* responded to bait in the wind tunnel

Exp. time	*n*	Bait	Attractive index*	
			Wind speed 0.13 m/s	Wind speed 0.58 m/s
0900-1200 h	180	100 g 1-day tainted pork viscera	0.38(0.35 to 0.67)^{ab}	0.15(0.15 to 0.18)^{c}
	180	300 g 1-day tainted pork viscera	0.33(0.18 to 0.48)^{bc}	0.23(0.15 to 0.30)^{bc}
1300-1700 h	180	100 g 1-day tainted pork viscera	0.63(0.45 to 0.63)^{a}	0.60(0.58 to 0.65)^{a}
	180	300 g 1-day tainted pork viscera	0.52(0.47 to 0.57)^{a}	0.63(0.50 to 0.65)^{a}
	180	1 kg 1-day tainted pork viscera	0.28(0.27 to 0.30)^{bc}	0.53(0.50 to 0.58)^{a}

*Data are expressed as medians (range) of three independent experiments, and different letters deemed significantly (P<0.05).

Assessment suitable number of fly

To assess the number of fly used in the wind tunnel experiment, 60 flies (30 females and 30 males) or 200 flies (100 females and 100 males) were recruited in each experiment, using 300 g of 1-day tainted pork viscera as bait. The procedure was performed in the same manner as wind tunnel experiment. The experiments were carried out triplicates.

When assessing the suitable number of flies employed in the wind tunnel experiment, small number (n=60) yielded more attractive index than those high number (n=200) in both two wind speed set (P<0.05) (Fig. 9). In the same group of small number, although the attractive index was higher when wind speed set at 0.58 m/s, but no significant difference was found in comparison with the wind speed set at 0.13 m/s.

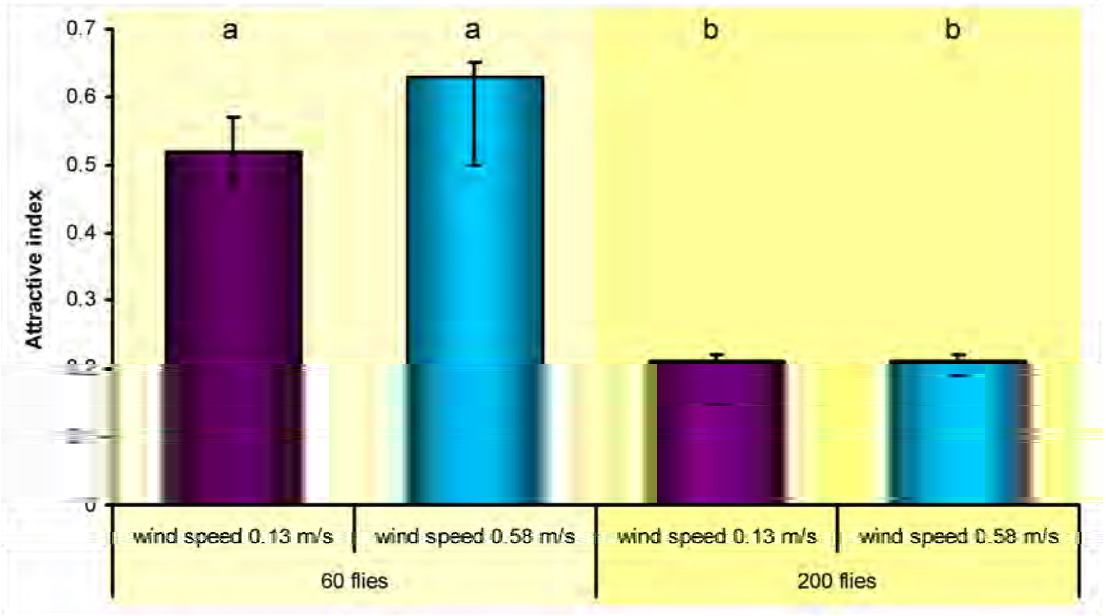


Fig 9. Suitable number of fly used in wind tunnel that responded to 1-day tainted pork viscera at wind speed of 0.58 m/s

Assessment light intensity with bait

The experiment was designed to determine the behavioral response of flies to different light intensity in the wind tunnel while having 1-day tainted pork viscera, the high attractive index, as bait. The procedure was performed in the same manner as wind tunnel experiment, with light intensity was adjusted as low (33.69 lux) and high (341.33 lux) light intensity as following groups:

Group 1 —33.69 lux (ceiling light), 0 W (light source)

Group 2 —33.69 lux (ceiling light), 10 W (light source)

Group 3 —33.69 lux (ceiling light), 100 W (light source)

Group 4 —341.33 lux (ceiling light), 0 W (light source)

Group 5 —341.33 lux (ceiling light), 10 W (light source)

Group 6 —341.33 lux (ceiling light), 100 W (light source)

The attractive index of *C. megacephala* response to 1-day tainted pork viscera when varying light intensity and light source was displayed in Table 12.

Table 12. Attractive index of *Chrysomya megacephala* response to 1-day tainted pork viscera when varying light intensity and light source

Light intensity	*n*	Light source (W)	Attractive index*
Low (33.69 lux)	180	0	0.42 (0.32 to 0.58)^{ab}
	180	10	0.33 (0.28 to 0.42)^{b}
	180	100	0.27 (0.21 to 0.33)^{b}
High (341.33 lux)	180	0	0.42 (0.33 to 0.43)^{ab}
	180	10	0.63(0.50 to 0.65)^{a}
	180	100	0.53 (0.50 to 0.58)^{a}

*Data are expressed as medians (range) of three independent experiments when wind speed set at 0.58 m/s, and different letters deemed significantly (P<0.05).

Assessment of concentration of the attractant-absorbed water (AW)

Three concentrations between weight (g) of the chopped viscera and volume (ml) of distilled water were formulated to optimal the condition of the aerobic extraction. According to the process of aerobic extraction with 500 ml of distilled water, 500 or 250 g of the chopped viscera were placed in the box, instead of 1kg of the chopped viscera, and were tainted in the incubator. Thereafter, the attractant-absorbed water (AW) at those three concentrations were tested their efficacy to attract *C. megacephala* [5-10 day-old VS well-fed] within the wind tunnel , with the wind speed being set at 0.13 m/s. Moreover, attractant-absorbed water (AW) after storage for 2-, 3- and 4-week-storage was also evaluated their efficacy. The result of this experiment was displayed in Table 13 and Fig. 10.

Table 13. Attractive index of virgin, 5-10 day-old, well-fed *C. megacephala* response to 100 ml of the attractant-absorbed water (AW) focusing of concentration and storage temperature in the wind tunnel at wind speed of 0.13 m/s

Attractant-absorbed water	Attractive index
Concentration* (ratio)	
- 1 kg of 1-day tainted pork viscera (2:1)	0.57(0.50 to 0.58)[a]
- 500 g of 1-day tainted pork viscera (1:1)	0.50(0.43 to 0.52)[a]
- 250 g of 1-day tainted pork viscera (1:2)	0.30(0.25 to 0.33)[b]
Storage temperature	
- Kept in -18 °C	0.42(0.37 to 0.48)[a]
- Kept in 4 °C	0.37(0.35 to 0.42)[ab]
- Kept in ambient temperature (20-35 °C)	0.33(0.20 to 0.33)[b]

Concentration* (ratio) = g of 1-day tainted pork viscera applied with 500 ml of distilled water
Data are expressed as medians (range) of three independent experiments at 25.0-30.0˚C and 36-77% relative humidity (*n*=1,080 flies)
Different letters deemed significantly (Chi square test; $p<0.05$)

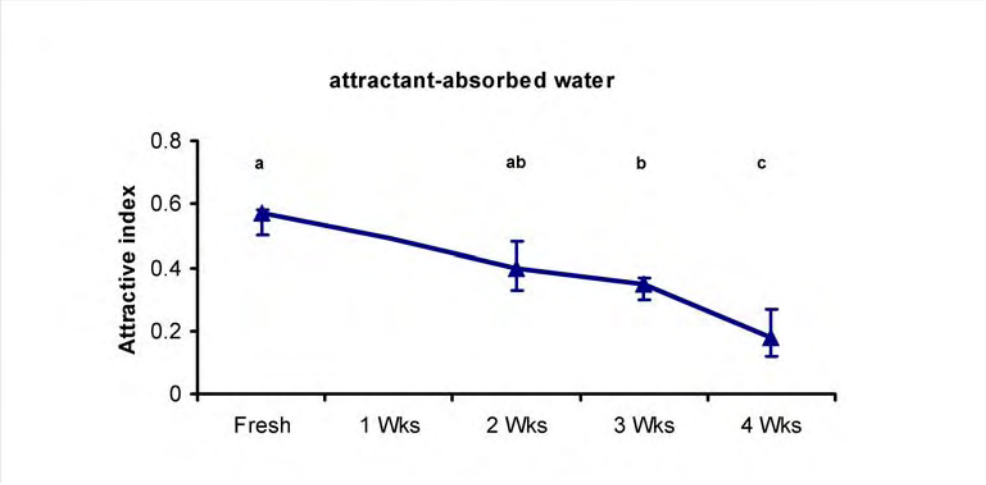


Fig 10. Attractive index of virgin, 5-10 day-old, well-fed *C. megacephala* response to 100 ml of the attractant-absorbed water (AW) after storage for 2-, 3- and 4-week-storage in the wind tunnel at wind speed of 0.13 m/s, 25.0-30.0 ˚C and 45-83 % relative humidity (*n*=720 flies)

Assessment of composition in the odor using GC/MS

The 1-day-tainted pork viscera has been evaluated for their composition using GC/MS, and the result compared with the database (Wiley version 7) showed in Table 14.

Table 14. Composition of compound analysed from the 1-day-tainted pork viscera using GC/MS

Retention time (min)	Compounds	% Relative
1.51	Ammonia	100.00
1.59	Methyl mercaptan	13.64
1.63	Ethanol	7.31
1.73	2-Heptanone	0.71
1.95	Propanol	0.43
2.16	2-*Butanone	1.79
4.19	Dimethyl disulfide	8.28
10.19	Dimethyl trisulfide	3.88
17.46	Indoline	0.26

Assessment of suitable bait in the field

Although the 1-day tainted pork viscera yielded the highest attractive index to lure *C. megacephala* in the laboratory, statistical analysis revealed non significant difference from that of 1-day tainted beef viscera (Table 4). In this regard, we used the 1-day tainted beef viscera to attract *C. megacephala* in the field trial, while the fresh beef viscera has been used for the house fly, with the result being shown in Table 15.

Table 15. Flies captured in the field using 1-day tainted beef viscera to attract *C. megacephala* and the fresh beef viscera to attract house fly, from traps at different study sites in Chiang Mai from May 2009 through May 2010

		Number of flies (% of total)			
Family	Species	Mueang Chiang Mai	Hang Dong	Mae Rim	All sites
Calliphoridae					
	Chrysomya megacephala	11,612	9,547	7,636	28,822
		(55.2)	(51.5)	(32.4)	(45.6)
	Other Calliphoridae	5,339	3,625	4,369	13,333
		(25.4)	(19.6)	(18.5)	(21.1)
Muscidae					
	Musca domestica	316	189	316	821
		(1.5)	(1.0)	(1.3)	(1.3)
	Other Muscidae	3,666	3,140	6,005	12,677
		(17.4)	(16.9)	(25.4)	(20.1)
Sarcophagidae		772	959	695	2,426
		(3.7)	(5.2)	(2.9)	(3.8)
Others		2,034	1,039	2,006	5,079
		(9.7)	(5.6)	(8.5)	(8.0)
Total		21,027	18,526	23,605	63,158

DISCUSSION

In the present study, the T-shaped apparatus of dual-choice wind tunnel was designed to simulate the natural situation of food searching of the insects. The trapezoid box connected to the main apparatus acts as a hood, thus the wind currents from both sides were physically mixed and drawn out by the role of hood-like box. We assumed that the odor molecules were mixed and dispersed inside the middle partition, thereby allowing flies can follow the odor plume to locate the odor-loaded partition correctly. The results performed using the dual-choice wind tunnel approved our hypothesis.

Wind is an important abiotic factor that may affect the behavioral responses of insects to olfactory stimuli [4]. The dispersion of odor in the wind current is dominated by turbulent diffusion. In this study, our results revealed that the wind speed affected significantly to behavioral responses of flies, with the number of responding flies toward olfactory stimuli greatly correlated to wind speed levels. At wind speed set at 0 m/s, flies showed upwind flight orientation in random direction, indicating flies responded both odor-free and odor-loaded partitions. Almost flies could not detect the odor-loaded partition properly, and this might be due to no wind as a cue for blowing the odor sources correctly. Generally, flying insects often follow odor plumes to find resources. Some insects may employ an “aim-and-shoot” strategy using mechanoreceptors before flight to determine wind direction [25]. In our investigations, when wind speed had the strong correlation to the number of responding flies. Higher wind speed could gradually reduce the number of fly in the odor-free partition. In this regard, wind speed should be necessary for orientation and navigation of *C. megacephala* to approach odor source rightly, which was consistent with previous reports in some insects, for example the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae) [26], whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) [25].

By considering the responding flies in the odor-free (blank) partition, significantly more number of responding flies in partition having wind speed set at 0-0.2 m/s was detected than those set at 0.3-0.5 m/s. Higher wind speed was set, making stronger olfactory signal; thus may be the reason why the number of responding flies that failed to locate the right odor source was decreased. However, the capacity of recognition toward olfactory stimuli or discrimination of olfactory signals for *C. megacephala* is unclear. In this regard, physiological study of *C. megacephala* concerning this aspect merits further investigation.

Our results revealed that the optimal wind speed inside the dual-choice wind tunnel for *C. megacephala* was 0.5 m/s, and this wind speed corresponds to clam environment in nature. This wind speed level (0.5 m/s) was the same as earlier studies regarding visual and olfactory cue interaction in blow fly, *Lucilia sericata* (Diptera: Calliphoridae) in the wind tunnel [27], and food odor learning of pupal parasitoid *Pimpla alboannulatus* (Hymenoptera: Ichneumonidae)

[28]. However, the optimal wind speed used to study behavioral response in wind tunnel varied in different groups of insect, such as mosquito, *Aedes aegypti* (Diptera: Culicidae) [14], and *An. gambiae* [15], was 0.1 m/s; for the midge, *Culicoides impunctatus* (Diptera: Ceratopogonidae) was 0.2 m/s [13]; for the fruit fly, *Drosophila melanogaster* (Diptera: Drosophilinae) was 0.4 m/s [12].

In insects, olfaction is a crucial sensory modality for controlling many aspects of behavior. Mate selection, food choice and navigation toward suitable oviposition sites all depend on a functioning sense of smell [29]. This study showed that olfactory stimuli could activate *C. megacphala* to demonstrate the behavioral responses in approaching the odor source and the upwind flight to locate the position of odor source. Adults of this fly have been reported in attracting a discrepancy food, e.g., human food, human corpse, animal feces or carrion [30-32]. The discriminant analysis of both screening in the rearing cage and particular testing in the wind tunnel clearly indicated that the tainted protein-based or animal-origin materials were more attractive than another, with the 1-day tainted pork viscera being the most attractive natural product for attracting *C. megacphala*. This was conformed to the previous report that the pork viscera had high efficacy as bait to attract *C. megacephala* in the field [5]. It is not clear why this fly species preferred the protein-based material. Other report indicated that the protein diet was required as an important role in ovarian development and mating behavior in anautogenous dipterans [33-35].

In our test, the screening method in the rearing cage is different in aim from that of the dual-choice wind tunnel. Based on Churdhurry et al. [22], the former method was a simple assay for rapid screening odor. Hall [11] has noted that fly behavior in short distance would probably be activated by visual, olfactory, tactile and thermal stimuli, all distribute to landing behavior on the target odor source. Conversely, investigation within the wind tunnel provided longer distance which only the windborne olfactory stimuli were available for activating behavioral responses, giving the most convincing evidence that an odor source is truly attractive to an insect, acting as positive responses in the tunnels [21]. The interesting aspect of our findings is that although the numbers of responding flies of both methods were different, they yielded the same responding trend, indicating that 24 kinds could attract >50% of tested flies and 1-day tainted pork viscera being the most attractive.

Investigation of flies against bait emitting olfactory stimuli has been conducted to study behavioral response in the laboratory, thereby providing beneficial information to be used in field research experiment, which is aiming to control fly population. Such investigations not only involving chemical factor of the bait, but biological factors of flies *per se* [7;18;22;36]. These biological factors included age, sex, physiological profile (mated versus virgin), as well as environmental factors, which were wind speed, light intensity, temperature, relative humidity

[4;16;37]. A variety of wind tunnels has been invented and employed to determine behavioral response of several insects [37-40]. As for testing in blow flies, Bunchu et al. [41] constructed and operated the “T-box dual-choice wind tunnel” to investigate the behavioral response of *C. megacephala*. However, limitation of using such model has arisen, e.g., large size of wind tunnel, material used could absorb the tested odor, no odor eliminator, small size of current electric ventilating fans. In the current study, we designed, constructed and operated the new wind tunnel model to improve its behavioral response efficiency. The main components — stimulus partition, trapped partition and release partition — were made of transparent sheet glasses with aluminium frame, which is light weight and could separate each partition independently, allowing to clean easily and dispose of trapped odor. Additionally, the transparent sheet glasses allow researcher to observed flies reside in each partition. Adjustment of wind speed could accomplished in variable options by switching the voltage of the fans, and the wind speed 0.93 m/s was attained, instead of 0.5 m/s, the highest wind speed, of the previous model. Regarding the cleaning of odor contamination from previous experiments, filtering and activated charcoal filter were used to remove emitting from the bait. Furthermore, using two light sources — ceiling light and light source — would control the appropriate light intensity within the wind tunnel.

The result also provided the valuable data regarding suitable time for behaviorally response of *C. megacephala* in the wind tunnel. The attractive index displayed the strongly response during 1300-1700 h in the afternoon time, correlating with the time to obtain a great number of this flies in the field survey [7;42]. The reason for this appearance of fly activity in Thailand is still unknown; however, it may be hypothesized from both fly and environmental factors. For the former factors, *C. megacephala* is synanthropic behavior, flies may have searching activity in the afternoon time. Regarding the latter factors, the organic materials produced by humans was continuously decomposed, thus alluring flies to come up. Additionally, the gradually increase temperature and decreasing relative humidity of the natural environment may enhance fly activity. Our results on the response to temperature of *C. megacephala* agree with a study by Ngoen-klan et al. [43] that higher number of flies correspond with higher temperature, but not with relative humidity. Nevertheless, these findings was in contrast with previous work [7] that this fly species had negative correlation with temperature, but positive correlation with relative humidity. In our experiments, temperature and relative humidity had been operated in the similar range in both morning (0900 – 1200 h) and afternoon hours (1300 – 1700 h). However, to clarify the influence of either intrinsic or environmental factors to observe fly responding, more experiments merit investigations.

Since the amount of baits can resulted in difference of fly’s behavioral response, it is vital to evaluate the quantity of bait used. Gibson and Torr [44] has demonstrated that number of

cattle can increase number of flies *Stomoxys* sp. attracted to host, but not for *Glossina* sp. As for evaluation of *C. megacephala* in this experiment, the use of 100 g or 300 g bait caused no significant difference in fly response in the wind tunnel, either at low wind speed (0.13 m/s) or medium wind speed (0.58 m/s). In contrast, increasing bait to be 1 kg caused lower response of flies, in particular at the low wind speed. Although it is yet unclear how small amount of bait yielded higher response, we assume that 100 g or 300 g of bait had the air-exposed area at the top more thoroughly on petri dish having diameter 14 cm. Despite its large amount of bait (1 kg), the air-exposed area at the top was similar at the surface area of diameter 14 cm, thus enabling lower releasing rate of odor emitting and then causing lower response of flies. This assumption was rely largely on the experiment of Becher et al. [45] that increasing surface area of bait by reducing its size until sprayed extract yielded more behavior response in the fruit fly, *Drosophila melanogaster*, than the authentic one. Our finding could also be important for developing the suitable amount baits for fly control in the field areas.

Behavioral investigation in the wind tunnel within the 10-min period after releasing flies into the release partition revealed that either 60 or 200 flies move forward to 1-day tainted pork viscera at bait. However, as time pass by, flies moved both forward and backward at the trapped partition, either with or without bait to find landing site. At the end of 1 h experimental period, flies in the group of 60 still landed at the bait-side; in contrast with flies in the group of 200 moved backward into the release partition and trapped partition had no bait, thus yielding lower attractive index in the latter group (see Figure 3).

In the experiment of bait-free in trapped partition, *C. megacephala* still moved from the release partition into the trapped partition. This phenomenon was also occurred in other insects which were tested in the choice assay [4;15;46]. The clean air which was devoid of odor or attractant may stimulate olfactory receptor neurons in insects, thus causing flies can move to the upwind stimulator [47], by which wind speed did not had any effect in the behavioral response of flies.

Wind speed is one of the prime factors operated in the wind tunnel for conveying odor and/or attractant molecules to reach receptors of flies. Many experiments have shown that the appropriated wind speed used triggered more fly response [4;44]. Stopped or low wind speed produced only small, if any, no trigger into the long distance, but only in the short distance [16]. However, the over wind speed may cause deformity or structure of the odor and/or attractant molecules, such as occurring in the odour plume, thereby reducing fly response [4]. Geier et al. [37] reveals that mosquito, *Aedes aegypti*, greater moves more to lactic acid which had filamentous feature than the homogenous feature. Experiment conducted in fruit moth, *Grapholita molesta* indicated that decreasing amplitude of odour yielded lower insect response (Baker and Haynes, 1989). Furthermore, over wind speed may directly affect insect flying,

particularly small or delicate one, as previously reported in the aphid parasitoid *Aphidius nigripes* [16], seeming that bait produce no attraction efficiency. On the other hand, insect may change its behavior in response to wind speed as well. In potato aphid, *Macrosiphum euphorbiae*, wind speed >2 m/s cause males to change flying behavior response to female pheromone by walking [46]. As for *C. megacephala*, our results indicated 0.58 m/s of wind speed yielded the most attractive index in the wind tunnel against 1-day tainted pork viscera as bait, which was similar to 0.5 m/s of this species [41] or other blow fly *Lucilia sericata* [27].

Our results revealed that 5-10 day-old *C. megacephala* response more to 1-day tainted pork viscera when having high light intensity than that low light intensity. This phenomenon was correlated with *C. megacephala* [41] and *Calliphora vicina* [48]. However, this finding was in contrast with some reports indicating that light intensity had minimal effect for behavioral response of diurnal active flies. Work published by Burkett and Butler [38] demonstrated that light, as the attractive source, did not attracted to mosquito *Aedes aegypti* and *Aedes albopictus*; whereas, could more attract to *Culex nigripalpus*, the nocturnal species. Similar finding was observed in *Culex quinquefasciatus*, which had active peak at night [49;50]. However, diurnal flies still required light for landing purpose [38]. Absence or minimal light intensity has been reported to cause stop or minimal response to bait, based on the response to flowers of walking thrips, *Frankliniella occidentalis*, the diurnal active species. In olfactometer and wind tunnel experiments, the lacking of light yielded lower response than those having light. However, the presence or absence of light did not have any effect for elderly insect [18].

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OUTPUT จากโครงการวิจัยที่ได้รับทุนจากสกว. และ สกอ.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

1.1 Bunchu N, Sukontason KL, Olson JK, Kurahashi H, **Sukontason K**. Behavioral responses of *Chrysomya megacephala* to natural products. Parasitology Research 2008;102:419-429.

Impact Factor ปี 2010 = 1.812

1.2 Siriwattanarungsee S, Sukontason KL, Olson JK, Chailapakul O, **Sukontason K**. Efficacy of neem extract against the blowfly and housefly. Parasitology Research 2008;103:535-544.

Impact Factor ปี 2010 = 1.812

1.3 **Sukontason K**, Bunchu N, Chaiwong T, Moophayak K, Sukontason KL. Forensically important flesh fly species in Thailand: morphology and developmental rate. Parasitology Research 2010;106:1055-1064.

Impact Factor ปี 2010 = 1.812

1.4 Ngoen-klan R, Moophayak K, Klong-klaew T, Irvine K.N., Sukontason K.L., Prangkio C, Somboon P, **Sukontason K**. Do climatic and physical factors affect populations of the blow fly *Chrysomya megacephala* and house fly *Musca domestica*? Parasitology Research 2011; in press.

Impact Factor ปี 2010 = 1.812

1.5 Moophayak K, Sa-nit S, **Sukontason K**, Vogtsberger R.C., Sukontason K. Morphological descriptions for the identification of *Hypopygiopsis tumrasvini* Kurahashi (Diptera: Calliphoridae). Parasitology Research 2011; in press.

Impact Factor ปี 2010 = 1.812

1.6 Moophayak K, **Sukontason K**, Kurahashi H, Vogtsberger RC, Sukontason KL. Improvement of wind tunnel for assessment behavioral response of flies. คาดว่าจะส่งตีพิมพ์ใน Parasite & Vector ขณะนี้อยู่ระหว่างการเตรียมต้นฉบับ

1.7 Moophayak K, **Sukontason K**, Chailapakul O, Kurahashi H, Vogtsberger RC, Sukontason KL. Attractant-dissolved solutions from pork viscera to attract blow fly *Chrysomya megacephala* (Diptera: Calliphoridae) ขณะนี้อยู่ระหว่างการเตรียมต้นฉบับ

หมายเหตุ Reprint ผลงานวิจัยเรื่องที่ 1-5 อยู่ในภาคผนวก

2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงสาธารณะ

มีการสร้างเครือข่ายความร่วมมือกับหน่วยงานอื่นคือ

- ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
- ภาควิชาภูมิศาสตร์ คณะสังคมศาสตร์ มหาวิทยาลัยเชียงใหม่
- Department of Biology, Midwestern State University, USA

- Department of Geography and Planning Department and Center for Southeast Asia Environment and Sustainable Development, Buffalo State, State University of New York, USA
- Department of Medical Entomology, National Institute of Infectious Diseases, Japan

- เชิงวิชาการ

มีการผลงานวิจัยไปพัฒนาการเรียนการสอน ในกระบวนวิชากีฏวิทยาการแพทย์ ในระดับบัณฑิตศึกษา ภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ และมีการสร้างนักวิจัยใหม่จากโครงการนี้ โดยร่วมมือกับโครงการปริญญาเอกกาญจนาภิเษก คือ อ.ดร. นพวรรณ บุญชู, อ.ดร. รัชฎาวรรณ เงินกลั่น, อ. สรวิชญ์ อุปคุตป์, นายกิตติคุณ หมู่พยัคฆ์, นส. ธันวดี คล่องแคล่ว

3. การเสนอผลงานในที่ประชุมวิชาการ

- ไปเสนอผลงานวิจัยแบบปากเปล่า เรื่อง Morphology and developmental rate of *Parasarcophaga dux* (Diptera: Sarcophagidae) in Thailand" ในการประชุมวิชาการนานาชาติ The 16th European Society of Vector Ecology Conference 2008 ณ เมืองเคมบริดจ์ ประเทศสหราชอาณาจักร วันที่ 25-28 มีนาคม 2551
- ผลงานวิจัยถูกนำเสนอแบบโปสเตอร์ เรื่อง A dual-choice flight tunnel and a detection system for studying behavioral responses of blow fly, *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) to airborne olfactory stimuli ในการประชุมวิชาการนานาชาติ "The Second Internaltional Forum for Sustainable Management of Disease Vectors" ณ กรุงปักกิ่ง ประเทศสาธารณรัฐประชาชนจีน เดือนธันวาคม 2551
- เข้าร่วมประชุมและนำเสนอผลงานวิจัยแบบโปสเตอร์ เรื่อง Construction of the Dual-Choice Flight Wind Tunnel with a Fly Number Detection System for Evaluating Appropriate Attractive Odor for the House fly, *Musca domestica* and Blow Fly *Chrysomya megacephala* ในการประชุม "นักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว." ณ โรงแรมฮอลิเดย์อินน์ รีสอร์ท รีเจนท์ บีช ชะอำ จังหวัดเพชรบุรี เดือนตุลาคม 2553
- เสนอผลงานวิจัยแบบโปสเตอร์ เรื่อง Responses of blow fly, *Chrysomya megacephala*, to odour-dissolved solutions in laboratory and field conditions ในการประชุม "Joint International Tropical Medicine Meeting 2010 ณ โรงแรมเซนทารา กรุงเทพฯ วันที่ 1-3 ธันวาคม 2553

4. ตำรา 1 เล่ม

คม สุคนธสรรพ์, กาบแก้ว สุคนธสรรพ์. แมลงวันหัวเขียวที่มีความสำคัญในราชอาณาจักรไทย. เชียงใหม่: กู๊ด-พริ้นท์ พริ้นท์ติ้ง; 2553. 494 หน้า. ISBN 978-974-672-523-1