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Spatial releasing properties and mosquito repellency of cellulose-based beads containing essential oils and vanillin

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A~~BSTRACT~~bstract

Porous cellulose beads (Viscopearl) manufactured from wood pulp can provide gradual-release action for aromatic substances. Here we immersed lemongrass oil, xanthoxylum oil, and vanillin to apply mosquito repellents. The volatiles from this Viscopearl were analyzed to obtain information for quality control (QC) or specification using a GC–MS, and its standard compounds were determined as linalool, geranial, neral, and vanillin. In a test using a 20 L chamber, it was confirmed that their constant amounts were released continuously and they did not be affected by light. In another monitoring test for 47 days on the Viscopearl equipped with a commercialized air conditioner operated for 8 ~~hours~~h every day under indoor conditions, the amounts of all released components increased in proportion to the open number of the pores on the module, which is a housing case containing the Viscopearl. In addition, the concentration of 8 major components including 3 standard QC compounds (linalool, geranial, and neral) in a repellency test room depended on both the ventilation and elapsed time. The vapor from the module did not affect main 7 plastic units of the air conditioner, whereas the Viscopearl equipped with an air conditioner showed good mosquito repellency under test chamber and room conditions that temperature, relative humidity, and carbon dioxide concentration were controlled at 24.8~~-~~–25.4 °C, 63.8~~-~~–65.7%, and 708~~-~~–1383 ppm, respectively. Therefore, the plant essential oil-based repellent Viscopearl has high potential to be used as human protective agent against indoor mosquitoes by applying it to air conditioners.

Keywords: ~~a~~Air conditioner; ~~r~~Repellent module; ~~c~~Controlled release; Viscopearl formulation; ~~x~~Xanthoxylum oil

Introduction

There are 41 genera of mosquitoes and approximately 3~~,~~500 species in tropical and subtropical regions (Ghosh et al., 2011). The most endemic disease among various diseases transmitted by mosquitoes such as *Culex* (Japanese encephalitis, west Nile, chikungunya), *Anopheles* (filariasis, malaria) and *Aedes* (chikungunya, dengue fever, Zika virus) is malaria. In South Korea, since malaria occurrence by *Plasmodium vivax* was firstly reported in 1993, less than 500 people have been infected after 2000 year and the malaria has been currently managed under elimination phase (KCDC, 2016). However, globally estimated 3.2 billion people in 95 countries and territories are still at risk of being infected with malaria and 1.2 billion are at high risk (WHO, 2017). Therefore, the dependence on mosquito prevention such as the use of a mosquito repellent for personal protection could be the best strategy comparing to the cure of mosquito borne diseases. Most insect repellents generally work by forming a protective vapor barrier avoiding a biting arthropod from direct contacting with the human skin or fabric surface (Nerio et al., 2010; Mandal, 2011).

Commercially successful mosquito repellents are often manufactured using synthetic chemicals such as DEET (*n,n*-diethyl-3-methylbenzmide), icaridin, or PMD (*p*-menthane-3,8-diol), which are nonbiodegradable and may lead to the high burdens to the environment and the unacceptable side effects (Antwi et al., 2008; Solomon et al., 2012). Although they are effective, the renewed interest on alternative repellents with the increased concern on human safety drove the development of the plant origin repellent products. Several plant essential oils including citronella, lemon eucalyptus, and catnip due to their relatively low toxicity, acceptable efficacy, and customer need have been registered to the US Environmental Protection Agency. These oils are required in large amount, which may cause irritant effects, to be effective owing to the evaporation of their high vapor pressure. One reasonable way in order to the bottle neck of the volatile plant repellents is to continuously evaporate them without directly excessive contact with skin. In addition, the most crucial phase in commercially manufacturing a mosquito repellent is how to control its quality. Generally, mosquito repellents applying to human skin or fabric surface have to pass through some specific tests ensuring that they are consistent at different time points in the manufacturing process, show effective over a long storage period, and also safe in allowed use. However, there are not appropriate specifications for a repellent module intended to be equipped to an air conditioner. In previous study, the repellency of the Viscopearl formulation, which is composed of lemongrass oil, xanthoxylum oil, and vanillin and is studied for its application to a repellent module, against *Aedes aegypti* females was determined (Kim et al., 2012).

In the present study, some approaches to develop a mosquito repellent Viscopearl formulation and to apply its practical uses in an air conditioner were designed. The change in composition of the prototype (Kim et al., 2012) to overcome the weak solubility of vanillin, the chemical analysis of the Viscopearl formulation prepared from this study and specification set of standard components for its quality control, the determination on released amount of the Viscopearl itself and several standard components into air, and the difference in releasing patterns of the Viscopearl equipped to an air conditioner with or without ventilation under a chamber and a room conditions were evaluated. These parameters are important in the quality measures of the Viscopearl characteristics as well as for better consumer acceptance.

Materials and methods

Essential oils and chemicals

Lemongrass oil and xanthoxylum oil were purchased from Berjé (Bloomfield, NJ) and Seema International (Delhi, India), respectively. Camphene, geranyl acetate, geranial, limonene, linalool, myrcene, verbenol, caryophyllene, geraniol, vanillin, and *α*-terpineol were purchased from Sigma-Aldrich (St Louis, MO). Citronellal and methyl cinnamate were purchased from Fluka (Buchs, Switzerland), and *β*-phellandrene and 4-nonanone were purchased from TCI (Tokyo Chemical Industry, Tokyo, Japan). Neral (*cis*-citral) was supplied by Korea Forest Research Institute (Seoul, Korea). All other chemicals were of reagent grade and available commercially.

Mosquito culture

Aedes aegypti colony was originally obtained from the National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul, Korea, in 1999, and was reared in the laboratory without exposure to insecticides. Adults were maintained in screen cages with a 10% sucrose solution soaked on cotton located on top of the cage. Mosquitoes were allowed to feed on the blood of live mice for complete reproductive cycle.

Larvae were kept in plastic trays (25 × 35 × 5 cm) containing 0.5 g of sterilized diet (40-mesh chick chow powder and yeast, 4:1 wt/wt). The rearing room was maintained at 27 ± 1 °C, 65–75% RH with a photoperiod of 12:12 h (L:D).

Viscopearl formulation

By the previous study (Kim et al., 2012), repellent activity of Viscopearl formulations containing the mixtures of essential oils and vanillin composed of 1:1:1 (vol/vol/wt) was evaluated in in-door conditions. However, in this study, the porous cellulose beads (10 g) composed of Viscopearl carrier (6 g) supplied from Rengo company (Tokyo, Japan) and the mixture (4 g) of lemongrass oil, xanthoxylum oil, and vanillin (8.5:8.5:3.0, vol/vol/wt) were prepared (Fig. S2). Two repellent modules (21.3 × 2.9 × 0.7 cm^3) (Fig. S2) containing the beads (10 g) was equipped with inner side of a commercialized air conditioner (LS-107CS model, LG Electronics). The module has 42 pores (2 mm diameter) on its upper side (Fig. S2).

GC–MS analysis and determination of standard components for quality control of the Viscopearl formulation

Chemical analyses of the Viscopearl formulation (lemongrass oil:xanthoylum oil:vanillin = 8.5:8.5:3.0, vol/vol/wt) were performed on an Agilent 6890 N (Palo Alto, CA, USA). Helium, the carrier gas, was used at a flow rate of 1.0 ml/min. The amount of sample injected was 0.2 µl in split mode (30:1). The injector temperature was set at 250 °C. The GC column was DB-5MS stationary phase (60 m × 0.32 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). The GC oven temperature was initially maintained at 40 °C for 4 min, then increased 6 °C per min to 250 °C and maintained for 4 min. GC–MS analysis of the formulation was performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) by Agilent (Palo Alto, CA, USA) 6890 Plus gas chromatograph equipped with a 5973 mass selective detector quadrupole mass spectrometer. The mass spectrometer was run in the electron impact (EI) mode with electron energy at 70 eV. The mass spectrometer was operated in full scan mode between 35 and 700 amu. The components of the Viscopearl formulation were identified by Kovats indices, comparison of the mass spectrum of each peak with those of authentic samples in a mass spectra library (The Wiley Registry of Mass Spectra 2001 Library Data, sixth ed.), and confirmed by comparison of retention times obtained by GC with those of authentic samples and an earlier report (Adams, 2001. Essential oil components by Quadrupole GC/MS. Allured Publishing Corp., Carol Stream, IL, USA). From this analysis, 4 standard components for quality control of the Viscopearl formulation were determined.

Analytic 20 L-chamber test

The Viscopearl was put into a 20 L-stainless chamber (Fig. S1) and the released amount of 4 targeted or standard components based on elapsed time was monitored every day for one week according to the Korea Standard Method for Indoor Air Quality Test (Korean Standards Associations, 2004) on the determination of the emission of volatile organic compounds from building products and furnishing. The chamber was supplied with clean air retained 50 ± 5%, R.H. and the air change rate in the chamber was 0.5 ± 0.05 times per 1 ~~hour~~h. The indoor temperature was kept 25 ± 1 °C. The components and air (3.2 L) released from the Viscopearl were collected at a rate of 134 ml per min every day using the Tenex TA absorption tube (TA Stainless tube, Supelco, USA, 178 mm × 6 mm OD × 4 mm ID, 2,6-diphenylene oxide polymer). The concentration of the standard components released from the Viscopearl was determined using GC (HP6890N) and TDS (thermal desorption system, Gerstel, Germany)-GC/MSD (HP5975) (NICEM, Seoul National University) analyses. In addition, the photo degradation of the standard components (linalool, geranial, neral and vanillin) released from the Viscopearl in the analytic chamber by a light source (Krypton light DT-201, Taesung, Korea) was analyzed.

The sample tests were carried out 4 times every 2 ~~hours~~h for 8 ~~hours~~h. The TDS conditions as follows; helium as the carrier gas at a flow rate of 1.0 ml/min was used, the amount of sample injection was 0.2 µl in splitless mode, the temperature program of cooled injection system was initially hold at −30 °C for

5 min and increased 12 °C per min to 280 °C (holding 5 min), and also the desorption temperature program was initially hold at 30 °C for 3 min and increased 60 °C per min to 280 °C (holding 5 min). The transferline temperature was 300 °C.

Released amount from Viscopearl formulation

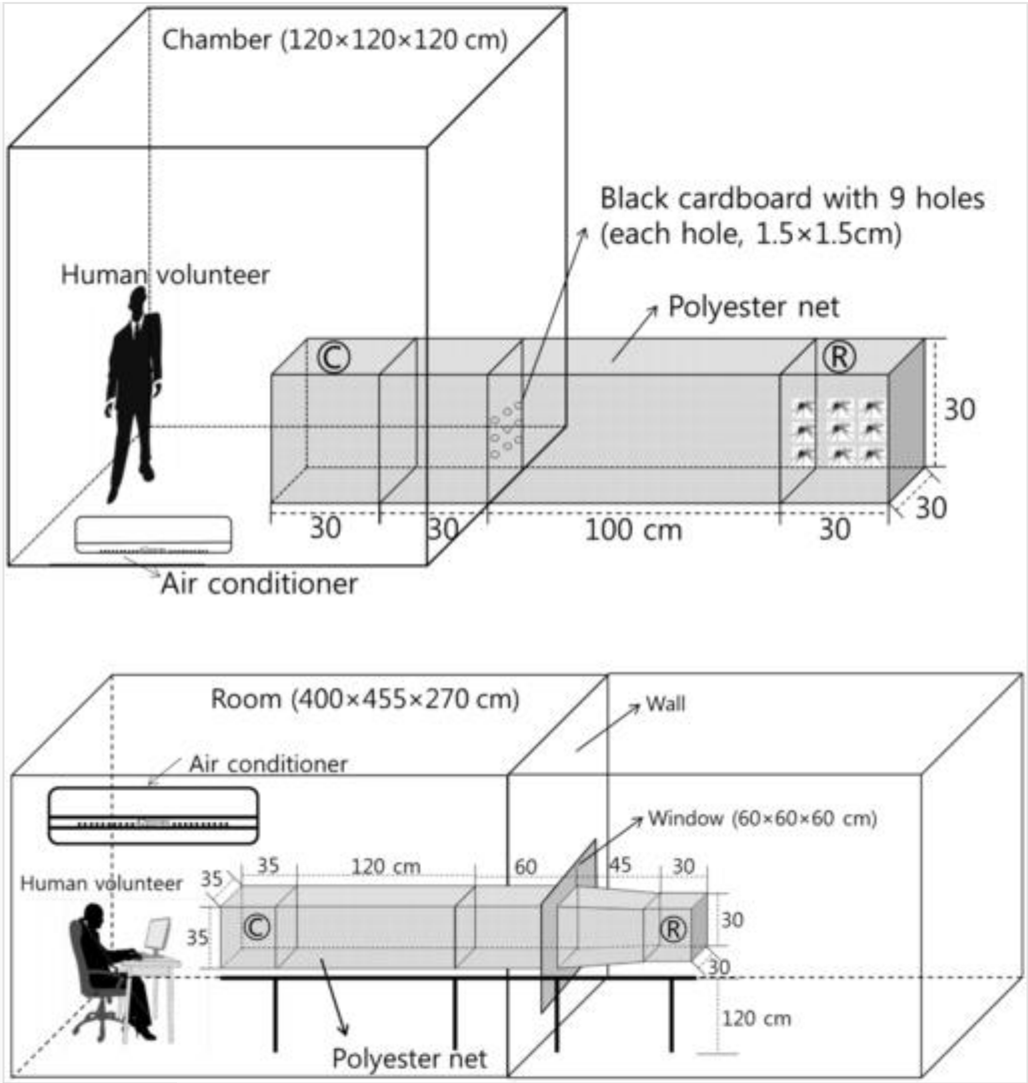
To evaluate the released amount of all components in the modules containing Viscopearl equipped with the commercialized air conditioner per unit hour, its 6, 10, and 20 pores were open under set conditions with a 25 °C, strong wind speed, and plasma mode. The tested air conditioner was operated for 8 ~~hours~~h every day under laboratory conditions. The loss amount released from 2 modules was weighted at 0, 4, and 8 ~~hours~~h every day after operating for 47 days.

Air sampling and analysis of 8 main components in a repellent test room

The concentration change of the two main standard components (linalool and neral) in the Viscopearl formulation in the repellent test room (4.0 × 4.55 × 2.7 m) without ventilation was monitored and in another indoor room (3.3 × 3.3 × 2.5 m), the spatial distribution properties of 8 main components (linalool, neral, geranial, limonene, 4-nonanone, β-phellandrene, camphene, and myrcene) released from the Viscopearl formulation were tested using an air conditioner at a ventilation of 0, 0.2, and 0.6. Ventilation means the speed (m/~~sec~~s) of wind made by ventilation fan in the indoor room. These tests are to determine the open pore numbers of the module under practical use of an air conditioner and the released amounts of the repellent module depending on a ventilation. Indoor air samples were collected every 0.5 h for 4 ~~hours~~h from 9 a.m. to 6 p.m. using a Tenex TA absorption tube (TA Stainless tube, Supelco, USA, 178 mm × 6 mm OD × 4 mm ID, 2,6-diphenylene oxide polymer) 1.0 and 1.2 m apart from the wall and floor, respectively. The tube was equipped to a portable pump (∑30, SIBATA Co, Japan) and air samples were collected 300 ml for 10 min. Each TA tube collecting approximately 3 L air was put into a zipper pack contained in ice box and then transferred to a laboratory for GC and TDS (thermal desorption system)-GC~~-~~–MS analyses. The operating mode of the air conditioners was the same as repellent testing conditions and the open number of pores on the repellent module was 8, 10, and 16 without ventilation, and 10 pores with ventilation, respectively.

The repellent tests of Viscopearl formulation

The repellency of the Viscopearl formulation (lemongrass oil:xanthoxylum oil:vanillin = 8.5:8.5:3.0, vol/vol/wt) against *Ae. aegypti* females was examined in a chamber (Kim et al., 2012) and a room. The chamber (1.2 × 1.2 × 1.2 m, Fig. 1A) has a rectangular gate (30 × 30 cm) in the front and bottom-center, and the hole was screened by a black card board with nine holes (each 1.5 × 1.5 cm). A mosquito cage (30 × 30 × 30 cm) containing non-fed *Ae. aegypti* females (aged 5~~-~~–8 d old) was connected to the rectangular hole and a collecting cage (30 × 30 × 30 cm) was placed inside the test chamber, which collected mosquito moved into the chamber after release. The collecting cage was also connected the rectangular hole and the testing compartments were like tunnel shape. Both the releasing and collecting cages were covered with polyester nettings. As the control, only a human volunteer was present in the chamber without any repellent components. One hundred nonblood-fed mosquitoes were used for the test. To determine the mosquito movement toward the upstream of human odors in the chamber, a tendency of the host location of mosquitoes was evaluated by mosquito flight movement into the chamber for 10 min when untreated Viscopearl. If the number of the female mosquitoes intruding into the collecting cage in the chamber was more than ten mosquitoes, repellent tests in the chamber were continued. In the chamber, a commercialized air conditioner (LS-107CS model, LG Electronics) was operated to promote the release of the Viscopearl components. The operating condition was strong wind speed, 25 °C, and plasma mode. Among 42 pores of the module, 10 ones were open to evaluate the repellency of the formulation. The number of females migrating into the chamber or the collecting cage was counted at 0.5, 1, 1.5, and 2 ~~hours~~h after the test started. All tests were carried out three times.



~~Figure 1.~~Fig. 1 Scheme of a chamber (A) or a room (B) test to determine the repellency of Viscopearl formulation containing lemongrass oil, xanthoxylum oil, and vanillin (8.5:8.5:3.0, vol/vol/wt) against *Aedes aegypti* females. In the room test, indoor temperature, relative humidity, and carbon dioxide concentration were also monitored for 4~~ hours~~h with or without ventilation, and air samplings were carried out every 30 min and then analyzed chemically using GC~~-~~–MS.

alt-text: Fig. 1

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Another test was carried out to evaluate the repellency of an air conditioner equipped with the modules in two testing rooms (4.0 × 4.55 × 2.7 m). The rooms were divided by a central wall with a window (60 × 60 × 60 cm). A mosquito collection cage (35 × 35 × 35 cm) was placed in the test room with the air conditioner and a mosquito releasing cage (30 × 30 × 30 cm) containing 250 *Ae. aegypti* females was positioned in the other room. The two cages were connected like a screened tunnel with 290 cm apart (Fig. 1B). The rooms can be ventilated and the temperature, relative humidity, and the concentration of carbon dioxide can be regulated by a control computer. The temperature and relative humidity sensors were positioned at the center of the rooms and beside the mosquito collecting or releasing cages. Carbon dioxide sensors (IAQ-Calc™ Indoor Air Quality Meter 7535, TSI, MN, USA) were set up the center of the rooms. The three condition factors were checked out every 0.5 ~~hour~~h for testing period. The air conditioner with repellent modules was operated with the same conditions as the chamber test for 1 ~~hour~~h in the test room, and the front of the releasing cage was tied with a rubber band to prevent the introduction of females into the test room. A human volunteer supplied a desk, a chair, and a laptop computer worked or done his matters freely in the operating room for testing period. After 1 ~~hour~~h, a rubber band was removed to allow free flight of females and the number of females introducing into the collecting cage was counted. The rooms were ventilated to remove a contaminated material in air for 0.5 h before and after every test. The temperature, relative humidity, and carbon dioxide concentration of the rooms were set up 25 °C, 60~~-~~–70%, and 500~~-~~–600 ppm, respectively. Mosquito repellency was calculated by a following equation: % repellency = (C-T/C) × 100, where C was the number of released females and T was the number of the females found in the chamber or room containing a volunteer.

Three healthy male volunteers from 20 to 40 yr old were recruited from volunteers living in Seoul and Suwon city, Korea. Before volunteers involved in this test, they were informed specific procedures and remedial arrangements for any discomforts that might occur. Once they understood this protocol, an informed consent form was provided for agreement. The protocol for this study received formal approval from the Institutional Review Board of Seoul National University (approval number: 1108/001~~-~~–002).

The side effects of Viscopearl components to plastic units of the air conditioner

The effects of the essential oil alone or the Viscopearl formulation (lemongrass oil:xanthoylum oil:vanillin = 8.5:8.5:3.0, vol/vol/wt) to seven plastic compartments (ABS HF380, HIPS SG970, PPM 540, HIPS 60HR, ABS XR401, ADP, and SAN) of the air conditioner were tested under room conditions. The compartments were prepared as a rectangular stick (1.5 × 5.0 cm) and each stick was dipped into a beaker (300 ml) containing lemongrass oil, xanthoxylum oil, and the Viscopearl formulation solution. The entrance of the beaker was closed by a parafilm under 24~~-~~–27 °C room and the discoloration, deformation, and surface melting of the parts were checked out 12, 24, and 48 ~~hours~~h after dipping. In addition, the effect by vapor phase released from the repellent modules to the seven plastic compartments was tested using an operating air conditioner set up 18 °C, strong wind speed, and plasma mode. All pores (42) of the module were open and the effects by vapor phase were checked 40 days after contacting. The suspended compartments in front of the air conditioner were directly contacted to exhausted air from it operated from 10 a.m. to 6 p.m.

Statistical analysis

Two-way ANOVA was used to analyze open pores, time and their interaction on spatial concentration (Table S2) and ventilation, time and their interaction on spatial concentration (Table S3) using SPSS Version 25 (IBM, New York, NY, USA). Experiments were carried out in triplicate and are presented as the mean ± standard error. The loss amount of repellent components in the modules data were analyzed by linear regressions using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

Chemical components of the Viscopearl formulation

By comparing Kovats indices, mass spectral data and retention times with authentic compounds using GC and GC/MS, components of the Viscopearl formulation were determined (Fig. S2, Table 1). Two isomeric monoterpene aldehydes, geranial (18.62%) and neral (14.71%), along with monoterpene alcohol (linalool 30.39%) and two mononocyclic monoterpenes such as limonene (6.96%) and *β*-phellandrene (4.08%), were the main components in the formulation. The other constituents were acyclic monoterpene (myrcene 0.94%), bicyclic monoterpene (camphene 0.31%), monoterpene alcohols (verbenol 0.36%, geraniol 2.84%, and *α*-terpineol 0.46%), monoterpene esters (geranyl acetate 1.84% and methyl cinnamate 2.55%), two sesquiterpenes (caryophyllene 0.92% and germacrene-D 0.43%), two hydrocarbons (6-methyl-5-heptene-2-one

0.44% and 4-nonanone 0.49%), and vanillin (13.28%). Based on this analysis, the standard components of the Viscopearl formulation for quality control of the repellent module equipped to an air conditioner were determined as linalool, neral, geranial, and vanillin.

~~Table 1.~~Table 1 The chemical composition of a mosquito repellent Viscopearl formulation containing lemongrass oil, xanthoxylum oil, and vanillin (8.5:8.5:3.0, vol:vol:wt) by gas chromatography coupled with mass spectroscopy (GC~~-~~–MS).

alt-text: Table 1

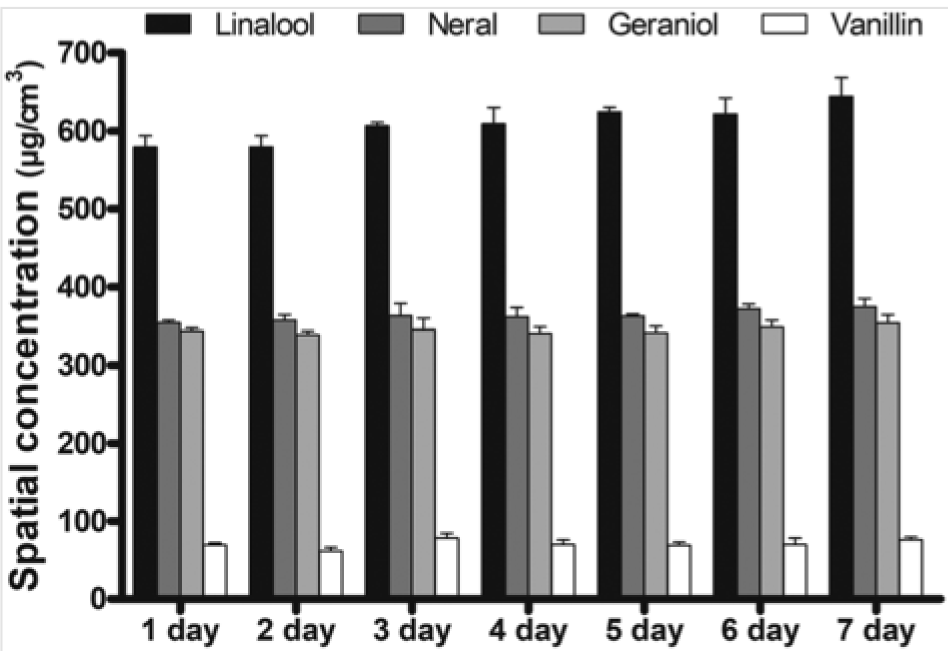
No.	RT[a], min	Compound	KI[b]	Relative composition ratio, %
1	8.445	Camphene	845	0.31
2	9.275	6-Methyl-5-heptene-2-one[c]	938	0.44
3	9.410	Myrcene	958	0.94
4	10.469	Limonene	1018	6.96
5	10.516	β-Phellandrene	1027	4.08
6	11.522	4-Nonanone	1052	0.49
7	12.275	Linalool	1082	30.39
8	14.233	Citronellal	1125	0.41
9	14.310	Verbenol	1136	0.36
10	14.657	α-Terpineol	1143	0.46
11	15.668	Neral	1174	14.71
12	15.915	Geraniol	1228	2.84
13	16.363	Geranial	1250	18.62
14	18.815	Geranyl acetate	1352	1.84
15	19.015	Methyl cinnamate	1363	2.55
16	19.262	Vanillin	1403	13.28
17	19.868	Caryophyllene	1442	0.92

No.	RT[a], min	Compound	KI[b]	Relative composition ratio, %
18	21.792	Germacrene-D[c]	1515	0.43

[a]Retention indices = van Den Dool and Kratz retention index (van Den Dool and Kratz, 1963) on DB-5 column, according to *n*-alkanes (C9–C17). Components were identified by co-injection with authentic standards.
[b]Kovats indices.
[c]Tentatively identified by mass library.

Releasing properties of 4 standard components in the Viscopearl using the 20 L-chamber

Air sample (3.2 L) containing components released from the repellent module in 20 L stainless chamber was collected every day for 1 week and spatial releasing amounts of 4 standard components were analyzed (Fig. S1). The spatial distribution mean amounts for 1 week of linalool, neral, geranial, and vanillin per unit volume (cm^3) were 609.29 ± 14.98-, 363.69 ± 8.13, 344.35 ± 8.55, and 70.53 ± 5.07 μg/cm^3 (Fig. 2). Their retention times were 31.02, 36.03, 36.83, and 41.71 min, respectively. The components were released continuously from the module. In another test to evaluate a change of the module components by light supplied for 8 ~~hours~~h into the chamber, no change of the components was detected. Especially, neral and geranial isomers did not be changed each other by light. These results indicate that the components are appropriate compounds for quality control of the Viscopearl.

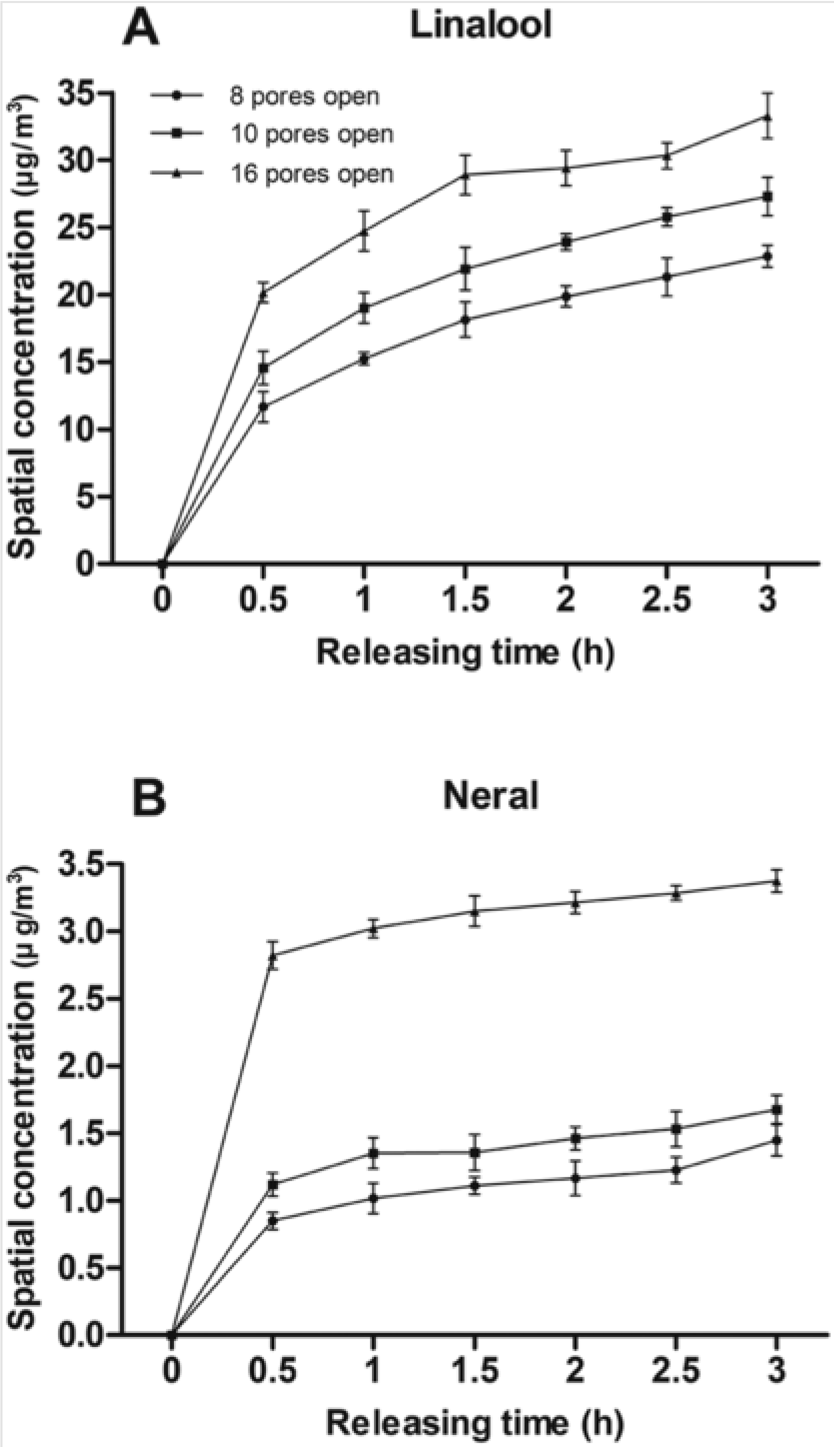


~~Figure 2.~~Fig. 2 The spatial distribution properties of 4 standard components (linalool, neral, geranial, and vanillin) released from the repellent module were monitored in a 20 L-test chamber.

alt-text: Fig. 2

Releasing properties of components in the Viscopearl formulation according to the number of open pores

The spatial distribution properties of the two main standard components (linalool and neral) in the repellent module were investigated based on the open number of pores (8, 10, and 16) under no ventilation in the test room (4.0 × 4.55 × 2.7 m). Air samples (30~~-~~*L*) were collected every 0.5 ~~hour~~h for 3 ~~hours~~h and they were analyzed using TDS-GC/MS. Linalool was released linearly for 1.5 ~~hours~~h after operating the air conditioner under 8, 10, and 16 pores were open, and then at 2 ~~hours~~h after operating, its releasing property showed logarithm pattern (Fig. 3A). At 0.5 to 3 ~~hours~~h after operating the air conditioner, the spatial concentrations of linalool increased from 11.68 ± 1.13, 14.57 ± 1.21, and 20.17 ± 0.74 μg/m^3 to 22.86 ± 0.80, 27.30 ± 1.42, and 33.31 ± 1.69 μg/m^3, when 8, 10, and 16 pores were open (Fig. 3A). The releasing pattern of neral was also similar with that of linalool. The spatial concentrations of neral increased from 0.85 ± 0.06, 1.12 ± 0.09, and 2.82 ± 0.10 μg/m^3 to 1.45 ± 0.12, 1.68 ± 0.11, and 3.38 ± 0.08 μg/m^3, when 8, 10, and 16 pores were open (Fig. 3B). Neral was released linearly from the module for 0.5 h after operating, but its releasing amount was decreased 1 ~~hour~~h after operating and was constantly released into indoor air (Fig. 3B). The released amount of linalool into air according to the open number of the module was a relatively irregular pattern, but that of neral showed proportional releasing property.



~~Figure 3.~~Fig. 3 The spatial releasing properties of linalool (A) and neral (B) when the 8 (●), 10 (■), and 16 (▲) pores of the repellent module was open without ventilation in a test room (4.0 × 4.55 × 2.7 m).

alt-text: Fig. 3

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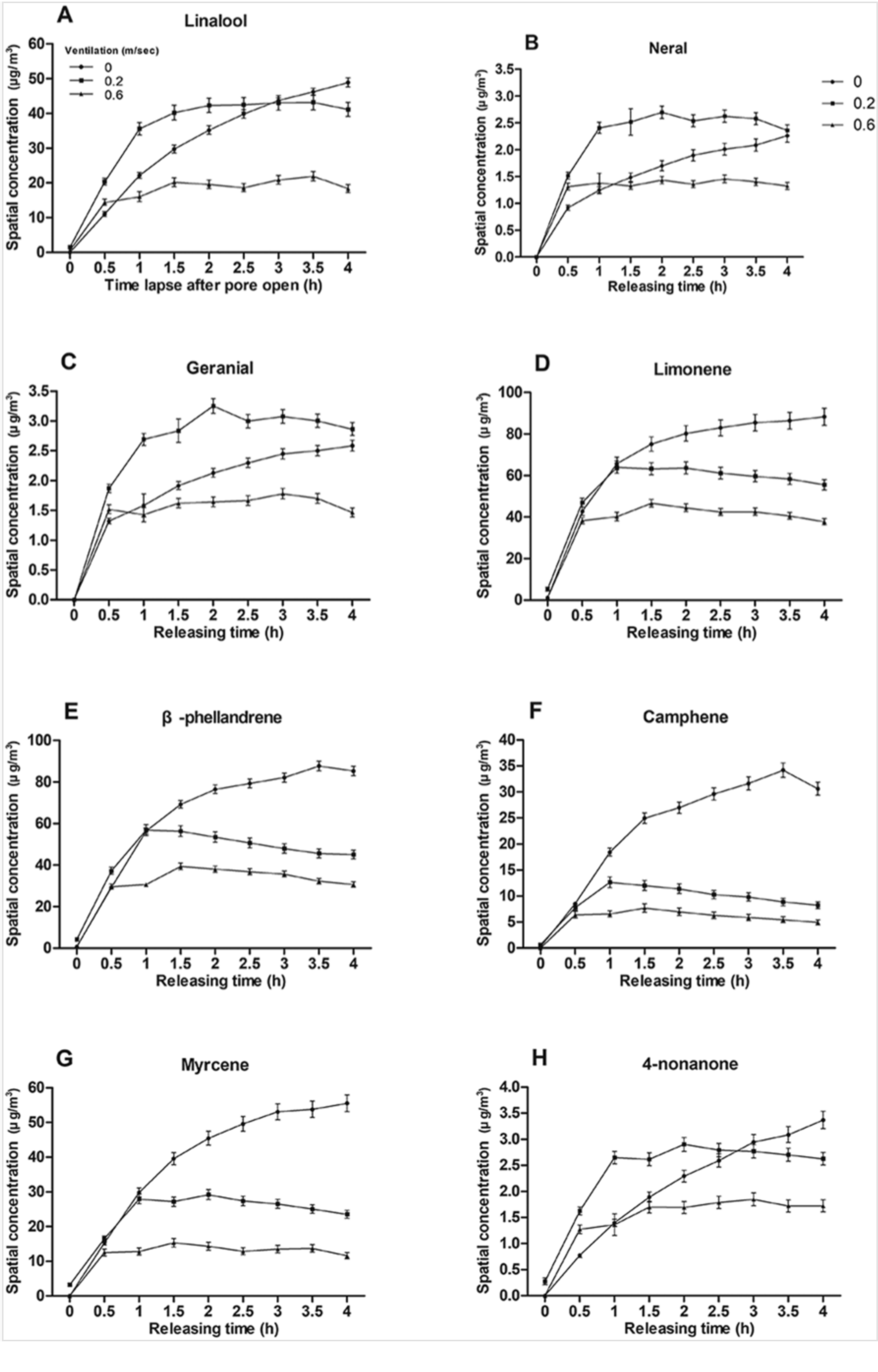
The spatial distribution properties of total 8 components (limonene, 4-nonanone, β-phellandrene, camphene, and myrcene) including 3 standard compounds (linalool, neral, and geranial) depending on a ventilation of 0, 0.2, and 0.6 times per 0.5 ~~hour~~h for 4 ~~hours~~h were evaluated in the indoor room (Fig. 4). Releasing logarithm patterns of 8 components in air samples (30 L) collected every 0.5 ~~hour~~h for 4 ~~hours~~h were similar with the results obtained from the chamber test and the no ventilation analysis. At a ventilation of 0.2 and 0.6 times per 0.5 ~~hour~~h, the concentration of the components depended on both the ventilation and elapsed time. Especially, β-phellandrene, camphene, and myrcene responded relatively sensitive to ventilation. Totally, the spatial distribution concentrations of the 8 components were 24.55 ± 0.95, 33.81 ± 1.39, 37.98 ± 1.36, and 39.61 ± 1.55 μg/m^3 at 1, 2, 3, and 4 ~~hours~~h after operating under no ventilation conditions (Fig. 4). However, in case of a ventilation of 0.2 times per 1 ~~hour~~h, their spatial concentration was increased from 25.59 ± 1.43 at 1 ~~hour~~h to 26.10 ± 1.28 μg at 2 ~~hours~~h, but 3 and 4 after operating, their concentration was decreased from 24.43 ± 1.19 to 22.67 ± 1.10 μg. In addition, the spatial distribution concentrations of the 8 components increased until 2 ~~hours~~h after operating (13.80 ± 0.80 to 16.01 ± 0.84 μg/m^3), but decreased from 15.44 ± 0.81 to 13.45 ± 0.70 μg/m^3 at 3 and 4 ~~hours~~h after operating under 0.6 ventilation (Fig. 4).

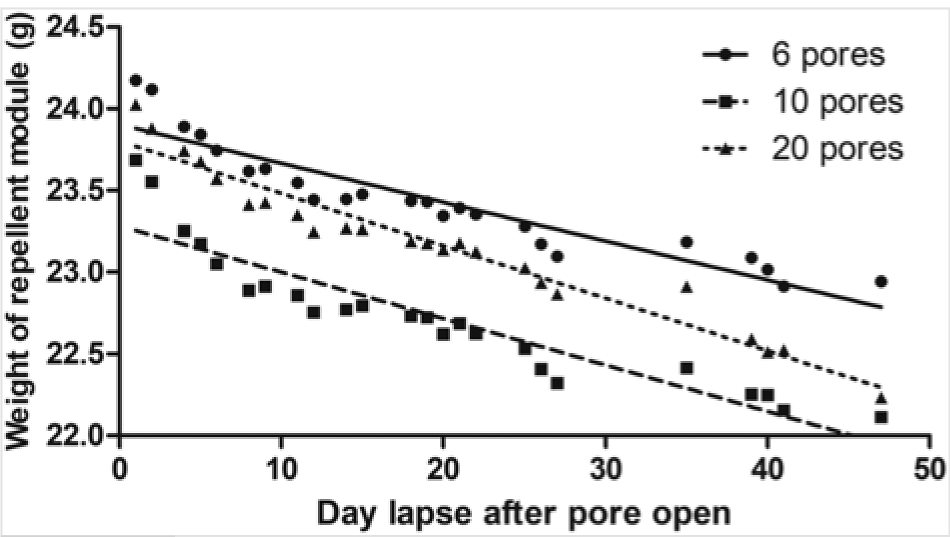

A
Linalool
Ventilation (m/sec)
0
0.2
0.6
Spatial concentration (μg/m³)
Time lapse after pore open (h)
B
Neral
Releasing time (h)
C
Geranial
D
Limonene
E
β -phellandrene
F
Camphene
G
Myrcene
H
4-nonanone

~~Figure 4.~~**Fig. 4** The spatial distribution concentration of 8 main components (A, linalool; B, neral; C, geranial; D, limonene; E, β-phellandrene; F, camphene; G, myrcene; and H, 4-nonanone) in the Viscopearl at a ventilation of 0, 0.2, and 0.6 times per 0.5 ~~hour~~h in an indoor room (3.3 × 3.3 × 2.5 m).

alt-text: Fig. 4

The loss amount of repellent components in the modules

By monitoring for 47 days on the air conditioners operated for 8 ~~hours~~h every day, the weight of the repellent module was decreased from 24.174 and 23.689 g to 22.942 and 22.114 at 47 days after operating under 6 and 10 pores open (Fig. 5). When the 20 pores were open, the loss amount of components in the repellent module equipped to the air conditioner was higher than that of the other cases. The loss amount of components in the repellent module showed a linearly decreasing pattern. When 6, 10, and 20 pores were open, the loss amount of repellent components in the module per day was 26.7, 34.1, and 70.6 mg indicating that its loss amount was depended on the number of its open pores. In addition, if linear slopes at constant checking points were compared, the amounts released for early 8 days and late 14 days were higher than those for middle test periods.



~~Figure 5.~~**Fig. 5** The loss amount of all components in the Viscopearl based on the open number of the pores on the repellent module equipped with an air conditioner were monitored. The components continuously decreased depending on the number of open pores, as shown in each regression line. The linear slopes of 6, 10, and 20 open pores were ~~-~~−0.0238, ~~-~~−0.0284, and ~~-~~−0.0321, respectively (R^2 = 0.87, 0.86 and 0.95, respectively).

alt-text: Fig. 5

Repellency of the Viscopearl formulation

The repellency of an air conditioner equipped with the Viscopearl formulation composed of lemongrass oil:xanthoylum oil:vanillin (8.5:8.5:3.0, vol:vol:wt) against *Ae. aegypti* females was tested in a chamber (1.2 × 1.2 × 1.2 m) and a room (4.0 × 4.55 × 2.7 m) conditions. In the chamber assay using 10 pores open among 42 pores of the modules, the air conditioner gave 78, 82, 83, and 80% repellency at 0.5, 1, 1.5, and 2 ~~hours~~h after operation against the mosquito females, respectively (Table S1). In addition, the repellency of it in a room, of which temperature, relative humidity, and carbon dioxide concentration were monitored during testing period. For 2 ~~hours~~h, the temperature, relative humidity, and carbon dioxide concentration of the test room were maintained 24.8~~-~~–25.4 °C, 63.8~~-~~–65.7%, and 708~~-~~–1383 ppm (Table S1). The repellency of the air conditioner was between 62 and 68%, and the activity was lower than in the chamber test.

The effects of Viscopearl components to plastic units of the air conditioner

Essential oil can be side effects such as deformation or corrosion to plastic products, so an effect by lemongrass and xanthoxylum oils alone contained in Viscopearl formulation as well as itself was tested using dipping in each oil and exposure methods to the vapor. When seven main plastic parts (ABS HF380, HIPS SG970, PPM 540, HIPS 60HR, ABS XR401, ADP, and SAN) composed of an air conditioner (LS-107CS model, LG Electronics) were dipped in a lemongrass oil, xanthoxylum oil, and the Viscopearl formulation solution, all plastic parts excepting for PPM 540 were melted, deformed, or eroded within 12 to 48 ~~hours~~h (Table 2). Although the side effects by the exposure materials were slightly different, the oils and formulation solution affected all plastic parts by dipping exposure (Fig. S4). In another test to evaluate the side effect by the vapor phase released from 42 pores of the repellent modules equipped to the air conditioner operating for 8 ~~hours~~h every day for 40 days, the seven tested plastic parts were not affected (data not shown).

~~**Table 2.**~~**Table 2** The side effects of main plastic materials (ABS HF380, HIPS SG970, PPM 540, HIPS 60HR, ABS XR401, ADP, and SAN) by dipping lemongrass and xanthoxylum oils, and the repellent Viscopearl formulation solution.

alt-text: Table 2

Material[a]b	12 h								
	Lemongrass oil			Xanthoxlyum oil			Viscopearl		
	M[a]	D	C	M	D	C	M	D	C
1	++[c]	++	++	~~-~~–	++	~~-~~–	++	++	++
2	++	~~-~~–	++	++	++	~~-~~–	++	~~-~~–	++
3	~~-~~–	~~-~~–	~~-~~–	~~-~~–	~~-~~–	~~-~~–	~~-~~–	~~-~~–	~~-~~–
4	++	~~-~~–	~~-~~–	++	++	~~-~~–	++	~~-~~–	~~-~~–
5	~~-~~–	~~-~~–	++	+	~~-~~–	~~-~~–	~~-~~–	~~-~~–	++
6	~~-~~–	~~-~~–	++	~~-~~–	~~-~~–	+	~~-~~–	~~-~~–	++
7	~~-~~–	~~-~~–	++	~~-~~–	~~-~~–	+	~~-~~–	~~-~~–	++
24 h									

Material[a]b	12 h								
	Lemongrass oil			Xanthoxlyum oil			Viscopearl		
	M[a]	D	C	M	D	C	M	D	C
	M	D	C	M	D	C	M	D	C
1	++	++	++	++	++	++	++	++	++
2	++		++	++	++	+	++		++
3									
4	++			++	++	+	++		
5			++	+		+			++
6			++			+			++
7			++			+			++
48 h									
	M	D	C	M	D	C	M	D	C
1	++	++	++	++	++	++	++	++	++
2	++		++	++	++	+	++		++
3									
4	++			++	++	+	++		
5			++	+		+			++
6			++			+			++
7			++			+			++

[a]Tested plastic materials. 1, ABS HF380; 2, HIPS SG970; 3, PPM 540; 4, HIPS 60HR; 5, ABS XR401; 6, ADP; and 7, SAN.
[b]M, melt; D, deform; C, corrosive.

[c]++, strongly, +, mildly, and -, not detected by visual observation.

Discussion

Many concerns on plant essential oils to use as repellent agents for personnel protection against mosquitoes have been focused. Lemongrass oil contains mainly neral and geranial as main components and linalool, citronellal, and β-myrcene as minor ones (Torres & Ragadioa, 1996; Schaneberg and Khan, 2002). Xanthoxylum oil derived from *Zanthoxylum armatum* seed contains linalool, limonene, and methyl cinnamate as main components, and especially the content of linalool is high (64.1~~-~~–44%) in Kashmir and Himalaya (Singh and Singh, 2011). These results are in agreement with this study that analyzed high content of neral and geranial. Although the repellency of geranial, neral, and linalool or plant essential oils against mosquitoes has been known (Leal and Uchida, 1998; Müller et al., 2009), their rapid evaporation is the most important factor in the loss of efficacy and some essential oils including lemongrass oil may cause skin irritation (Zhang et al., 2012). To prevent the direct contact of repellent oils with human skin and rapid evaporation, the research on the development of a formulation to reduce the rapid loss of plant essential oils into air is needed.

The use of encapsulated oil based-nanoemulsion or that of the mixture of *Cymbopogon* essential oil with 5% vanillin to reduce the release rate of plant essential oil gave considerable prolonged protection time (Tawatsin et al., 2001; Sakulku et al., 2009). Based on these previous studies, vanillin was used to improve the mosquito repellency in this study. The repellency of the lemongrass and xanthoxylum oils as well as synergic effects by vanillin addition against biting arthropods have been known (Kamsuk et al., 2007; Hieu et al., 2010; Baldacchino et al., 2013; Sritabutra and Soonwera, 2013) In addition, ointment and cream formulations of lemongrass oil in a topical application to an experimental bird showed repellency to *Ae. aegypti* adults (Oyedele et al., 2002) and an aerosol formulation containing 5 or 10% xanthoxylum oil-5% vanillin gave 100% repellency at 60-min postexposure in field tests (Kwon et al., 2011). In this study, an air conditioner equipped with the Viscopearl formulation composed of lemongrass oil, xanthoxylum oil, and vanillin showed good repellency under the chamber and room tests.

However, for the commercially stable production of a formulation containing volatile essential oils, the specifications for its quality control is a very crucial parameter because information on standard specifications of repellent products must be prepared to apply for the registration to authorities. The standard or reference compounds in the Viscopearl carrier were determined as geranial, neral, linalool, and vanillin by GC or GC~~-~~–MS because of their high content. This quality control management method focusing on the main ingredients such as linalool, linalyl acetate, and camphor contained in lavender oil was generally used in other products (Mori et al., 2002). The 4 standard components were released constantly from the Viscopearl for testing periods in the 20 L chamber tests. These results indicate that the Viscopearl beads containing volatile substances release the components with relatively constant ratio and order. In addition, the releasing amounts of the Viscopearl depended on both the number of the open pores on the module and ventilation conditions, although those of the released compounds were a little unstable at the ventilation of 0.2 times per hour. Releasing order of the components in the Viscopearl may be their differences in volatility due to different structural types such as acyclic, monocyclic and bicyclic monoterpenoids or adsorption strength. For example, vapor pressures of linalool and neral are 0.16 and 0.0913 ~~mm Hg~~mmHg at 25 °C, respectively. In a study on the emission characteristics of volatile organic compounds (VOCs) in five types of common furniture products using a chamber (5 m^3), the emission rates of VOCs were arranged in the order of aromatic, terpenes, carbonyl, paraffin, and halogenated paraffin (Ho et al., 2011).

Volatile compounds can be adsorbed to starch as well as cellulose, and one mechanism involved in the adsorption may be hydrogen bonding between hydroxyl groups of cellulose and polar groups of the volatile compounds (Dernovaya and Eltekov, 1988). Larger amounts of volatile compounds were desorbed at 50 °C than at 20 °C (Börjesson et al., 1994). In this study, the repellent composition containing volatile essential oils was absorbed into the Viscopearl, cellulose beads. Terpenes are emitted indoors by various building materials and consumer products and are often found at significant indoor concentrations (Baumann et al., 1999; Singer et al., 2006; Toftum et al., 2008). Especially, indoor volatile organic compounds including limonene, aldehydes, and aromatics are oxidized by ozone, hydroxyl radical, or nitrate radical, and photolysis is a strong

OH formation mechanism (Waring and Wells, 2015). In this study using 20 L chamber, the components in the Viscopearl were not deposed by the supply of photo source.

Studies to develop a controlled-release insect repellent formulation, which provides extended protection against mosquitoes, is safe and easy for topical use, and is also compatible with plastics or synthetic fabrics, have been done. Several film-forming formulations containing silicone and acrylate polymers or microencapsulated formulations showed extended repellency without direct relationship to DEET concentration (Reifenrath and Rutledge, 1983; Mehr et al., 1985; Gupta and Rutledge, 1989). In addition, conventionally burning plants or wood containing repellent compounds and more advanced methods such as mosquito coils, vaporizing mats, and aerosol spray cans have been used for providing relatively cheap and protecting several people at a time in a limited space. However, there was not any trial to help to protect human from mosquito biting by releasing repellent ingredients using air conditioner into the indoor. Because essential oils or volatile compounds often act at a vapor phase to mosquitoes and are effective for a relatively short protective time, these releasing properties of the Viscopearl may be very important in exerting constant repellency in a limited space. As expected, the Viscopearl, which is designed to retain high volatile ingredients for longer periods, equipped with an air conditioner showed good repellency against *Aedes* females under the chamber and room conditions.

Conclusions

In this study, the Viscopearl containing the mixture composed of lemongrass oil, xanthoxylum oil, and vanillin did not affect the plastic materials of the commercialized air conditioner via vapor exposure as well as gave good repellency. Therefore, the cellulose based Viscopearl by applying to appropriate air conditioner has potential to be used as repellent means against indoor mosquitoes.

Uncited references

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Torres and Ragodio, 1996

Van Den Dool and Kratz, 1963

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~~Appendix A.~~Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aspen.2018.12.024.

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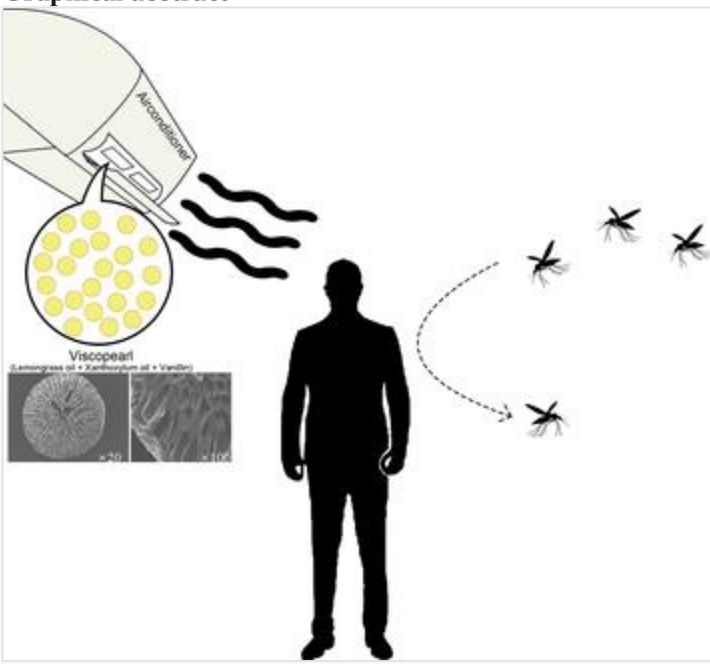
Graphical abstract



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Highlights

•Viscopearl was immersed with lemongrass oil, xanthoxylum oil, and vanillin.

•The standard compounds of Viscopearl were linalool, neral, geranial and vanillin.

•Concentration of compounds were depended on both the ventilation and elapsed time.

•Viscopearl equipped with air conditioner showed good mosquito repellency.

•Viscopearl could be used as a human protective agent against indoor mosquitoes.

~~Appendix A.~~Appendix A. Supplementary data

Multimedia Component 1

Supplementary material

alt-text: Image 1