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# Mosquito repellent fabric: Development and characterization of peppermint and garlic mixture finish on knitted fabric to examine mosquito repellency

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# ABSTRACT

Mosquito-repellent textiles are a part of protective textiles which help in protection from the species that are prone to cause diseases like malaria and dengue fever. This study explored the possibility of natural extract (alcoholic) from peppermint leaves, stems, and garlic cloves to use as a mosquito-repellent finish material on knit fabric. Accordingly, different concentration (5%, 15%, 25%, and 35%) of PGE (Peppermint Garlic Extract) solution was prepared and applied to the developed fabric using an exhaust dyeing process to assess the mosquito (Aedes Aegypti L.) repellency performance. Following WHO (World Health Organization) standard (cone bioassay) and a self-modified cage technique from literature survey, mosquito protection and repellency tests have been performed for characterization. The findings revealed that the PGE-treated fabric samples C (25% PGE) and D (35% PGE) had the highest mosquito mortality (50.00% and 76.67%, respectively) and repellency (78.6% and 85.6%, respectively) rates. Moreover, this study evaluated the prepared PGE formulations' shelf-life performance and colorfastness properties of PGE-treated fabrics, including the impact of washing cycles on the treated fabrics. There was no fungal growth, and the fabric showed excellent colorfastness properties. However, the efficacy of treated fabrics decreased with an increasing number of washes.

# 1. Introduction

Who is the biggest killer on the planet? No furious or giant animal, the minuscule mosquito is considered one of the deadliest animals in the world. Surprisingly, this tiny mosquito is responsible for more than one million deaths yearly. Mosquitoes are the most medically significant vector that transmits parasites, pathogens, and arboviruses such as malaria, filariasis, dengue, and chikungunya

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[1–3] Among these vectors, dengue is considered one of the most malignant viral viruses that has been rapidly transmitted to humans through a bite [4]. Most cases and deaths were reported in sub-Saharan Africa. However, Asia, Latin America, parts of Europe, the Middle East, and two of the Pacific countries are also affected due to mosquito-borne diseases [5]. In 2019, countries in all of these regions reported their highest number of dengue cases; the American region was 3.10 million, Bangladesh 1.01 million, Philippine 0.42 million, Vietnam 0.320 million cases, and Malaysia 0.131 million [4].

People working in the countryside, parks, forests, etc., may like to wear insecticide-finished clothing to protect themselves from blood-feeding arthropods, i.e., mosquitoes [6]. Therefore, it is compulsory to find a way to tackle such a problem, for example, a substance applied to skin, clothing, or other surfaces which discourages insects (and arthropods in general) from landing or climbing on that surface [7]. An effective way to control these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting humans [8–10]. A newly emerging field called mosquito repellent fabric, a basic human need, can provide the best way to tackle mosquito bites and knock down and repel mosquitos away when they come into contact with it [11,12].

Mosquito repellents are primarily categorized into two groups: chemical repellants and natural repellants. People initially applied mosquito repellents on their skin as lotion, which was effective only for a few hours. Furthermore, most of them can be harmful since they come in direct contact with the body [13]. Previous research found that synthetic antimicrobial agents are highly effective in preventing microbes' growth; however, they are non-eco-friendly and non-biodegradable [14]. For example, earlier, synthetic mosquitocides - DEET (N, N-diethyl-m-toluamide), permethrin, etc., have been chiefly used [6,12,15,16]; however, both these have toxicological profiles; cause tingling, burning, and itching on human skin [17]. Conversely, many natural plant-based extracts yield antimicrobial activity due to their diverse naturally available chemical composition [18]. In addition, plants and their extracts have been utilized as crucial sources of medicine for ages [19–21]. For instance, extracts from plant sources contain a wide range of chemically active ingredients (tannins, phenols, polyphenols, carotenes, quercetin, etc.) and can intervene and prevent several insects like moths, carpet beetle, mosquitoes, etc. [22]. Scientists work on natural ingredients, mint leaves [17], castor oil [7], fresh moringa leaf [23], neem and tulsi [17], sweet basil and eucalyptus [24], and marigold petals [25] to develop mosquito repellent cloth [26].

Two research groups developed finishing materials using Marigold (Tagetes erecta) flower petals applied on the cotton fabric. They investigated the performance against Culex quinquefasciatus (female) and undefined home mosquitoes, which showed 66.25% and 100% repellency, respectively [25,27]. In addition, Zayed et al. (2022) [28] found 98% repellency and a 95% death rate after 12 h of using an extract of *Citrus Sinensis* peel on the cotton fabric surface. Mint plants' ketone monoterpenes, including pulegone, carvone, and menthone are the most toxic to insects which are the most active [29]. A study stated that the presence of highly concentrated menthone and methyl ester acts as larvicidal and repellent [30]. Another research used medicinal plants like Neem (Azadirachta indica), Tulsi (*Ocimum tenuiflorum*) & Mint-leaf (Mentha), etc., on cotton fabric and analyzed their effectivity against the mosquito. Every extract showed sufficient repellency; Mint leaf was found to have 90% repellency. However, they reported a significant anti-mosquito efficiency loss after the ninth wash cycle [17]. Similarly, Gupta et al. (2017) [1] applied extracted Mint leaves directly on the fabric surface and showed almost 100% repellency. But during repeated washing, the coating was removed gradually.

Recently, researchers are also interested in Allium sativum L. as a possible tick-repellent source, especially in light of a report showing that soldiers exposed to ticks were protected against tick bites in the field by consuming garlic [31]. Garlic has been used for its medicinal properties for thousands of years and has a wide spectrum of actions; it is beneficial for its antibacterial, antiviral, antifungal, and antiprotozoal properties [32]. Even a study found that garlic's essential oil (EO) showed the ability of intoxication and necrosis in the larva, pupa, and adults of Tenebrio Molitor between 20 and 40 h after exposure [33,34] because of its compounds dimethyl trisulfide [33], dimethyl disulfide [35], diallyl sulfide, and 3-vinyl-[4H]-1,2-dithiin [34]. Peppermint oil is a flavoring of pharmaceuticals, is used for several purposes including dental preparations, mouthwashes, cough drops, alcoholic liqueurs, perfumery, soaps and detergents, and mosquito repellant [36]. Moreover, peppermint oil has proved to be an efficient larvicide and repellent against dengue vectors [30]. In addition, it is utilized as an environmentally friendly insecticide and pesticide for its capability to destroy various pests like wasps, hornets, ants, and cockroaches [37]. There is a growing interest in using botanical pesticides to decrease the use of chemical pesticides and avoid problems with insecticide resistance. Thomas & Callaghan, (1999) [38] used raw garlic and lemon peel extracts by crushing material in water. Both garlic and lemon were toxic to Culex pipiens mosquitoes; however, garlic was more persistent than lemon, with no significant differences in kill between new and approximately 4.5-day-old treatments. Similarly, Bassett, (1998) [39] identified that a mixture of garlic juice and hot pepper sauce repelled mosquitoes, but the repellent contribution of each of these components remained unknown. In another research, extracts of garlic (Allium Sativa) were toxic to the mosquitoes, with 90% of Culex larvae dying after only 8 h of exposure at 50 ppm [40].

From the previous literature review, it was found that most mosquito-repellent fabric-related studies use plant-based EO to replace synthetic repellents [5,41–45]. On the contrary, several studies have been done utilizing alcoholic or aqueous plant extract to implement on fabric surfaces that didn't show decent anti-mosquito durability after washing [17,27]. No study has been undertaken using an alcoholic combination of peppermint and garlic extract. Therefore, in this study, several investigations were done considering a few possible and renowned testing methods and knitted a cotton fabric following a unique design to find the best repellent performance. As a result, this experiment can provide a new class of sustainable and eco-friendly repelling textiles that provide protection against mosquito-borne diseases worldwide.

# 2. Materials and methods

## 2.1. Raw materials

100% cotton 30 Ne pre-treated (scoured and bleached) yarn was collected from the spinning lab of Textile Engineering College, Zorargonj, Chattogram, Bangladesh. As per the information from yarn manufacturer, the yarns have undergone a pre-treatment process namely scouring and bleaching. This pretreatment process typically involves the removal of impurities such as oils, waxes, seed hulls, soils, and pectin. Additionally, the process also enhances the whiteness of the yarn [46]. Peppermint (*Mentha piperita* L.) was collected from the campus garden of the National Institute of Textile Engineering and Research (NITER), Dhaka, Bangladesh and fresh garlic (*Allium sativum Linnaeus*) was purchased from the local market. For alcoholic extraction, 99.8% methanol (CH<sub>3</sub>OH), sodium-lauryl-ether-sulfate ( $C_{14}H_{20}NaO_5S$ ), and sodium benzoate ( $C_7H_5NaO_2$ ) were purchased from Techno Power Bangladesh.

## 2.2. Mosquitoes

Dengue virus carrier Aedes Aegypti L. mosquitoes were collected from the high-risk zone of Dhaka and were identified and separated from other mosquito types in the lab of NITER, Savar, Bangladesh. Before administering the experiment, collected mosquitos were kept in a large flask covered with a nylon net and kept starving from blood, sugar, and glucose for 4 h [24,47]. Fig. 1 represents an overview of PGE-treated fabric preparation.

# 2.3. PGE formulation preparation

Initially, garlic cloves and peppermint leaves were washed with distilled water to remove climatic impurities and left to dry. After that, garlic and peppermint were ground separately through a home grinder machine (Jaipan Home Grinder, India) and peppermint was mixed with garlic at a weight ratio of 3:1. The rational for this mixture is to test whether the addition of a small amount of garlic can improve the repellent performance, based on previous research by Mia et al. (2020) [17] where they tested only the effect of peppermint. By slightly increasing the concentration of natural ingredient in methanol compared to Mia et al. (2020) [17] study, we added 5 g of mildly ground peppermint and garlic with 80 mL of 99.8% methanol (1 g of ground peppermint and garlic mixture mixed with 16 mL methanol). The mixture was left for 24 h at room temperature (27 °C  $\pm$  2). After 24 h of extraction, 80 mL of PGE solution was collected by filtering, and pH 5.5 was measured. According to the study plan, the extracted solution's concentration was increased by evaporating to more than half of its total volume at a low heat (45–50 °C) and ended up at 30 mL.

### 2.4. Fabric preparation

### 2.4.1. Knitting

This study used a 30-inch cylinder diameter circular knitting machine (Santoni, China) with, a machine gauge of 24, and 90 feeders for knitting the honeycombed-shaped fabrics. The knitting parameters for producing the fabric is shown in Table 1 and diagrammatic notation of the fabric design, needle arrangement and cam arrangement design is shown in Fig. 2.



Fabric treatment through Exhaust dyeing method

Fig. 1. Overview of PGE-treated fabric preparation.

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# Table 1

Knitting parameters of study fabric production.

| Specifications   | Knit and Tuck loop  | Microstructural appearance |
|--|---|----------------------------|
| Creel arrangement<br>Yarn tension<br>Stitch length<br>Yarn count<br>Needle | 1,2,3,4,5<br>2-3 Centi Newton<br>2.57 mm<br>30 Ne<br>1 and 2 butt |                            |
|  |   | Front Back                 |
| Diagrammatic Notation  | Needle Arrangement  | Cam Arrangement            |



**Fig. 2.** Representation of diagrammatic notation, knitting needle, and cam arrangement (Adapted from 48). Note: CT=Cam truck; F=Feeder; K=Knit cam; T = Tuck cam.

# 2.4.2. Application of PGE formulation on fabric

Since the yarn was already pre-treated, a light soaping was conducted to remove impurities with a non-phosphate washing detergent (3.5 g/L) after knitting the fabric. The knitted fabric was subjected to dyeing using PGE solution with a material: liquor ratio of 1:7, utilizing an in-house infrared laboratory dyeing machine (TD130, China). At the stage of 30 °C temperature (Fig. 3), the fabric was loaded with a pre-planned PGE percentage (5%, 15%, 25%, and 35% refers to sample A, B, C, and D respectively), 60 g/L glauber salt, 0.01 g/mL Albatex DBC, and left to reach the temperature of 60 °C. At the beginning of 60 °C, soda (Sodium carbonate: Na<sub>2</sub>CO<sub>3</sub>) dosing was done. Then, the rest of the dyeing process was carried out in an alkaline medium for 60 min at the same temperature. When dyeing was completed, the dyed fabric was washed with regular mineral water, and then PGE dyed fabric was padded with 0.3 MPa, at a roller speed of 20 ms<sup>-1</sup>. Finally, the dyed fabric was dried at 100 °C for 5 min. Before starting the characterization of the PGE finished fabric's performance, samples were conditioned at 65% Relative Humidity (RH) and 25  $\pm$  2 °C temperatures for 24 h according to ASTM D1776/D1776M – 20 standard [49].



Fig. 3. Representation of fabric finishing over the time.

## 2.5. Assessment of shelf-life of PGE repellent formulation

The shelf life of a plant-based solution means the time expected to remain effective, free from damage, and stay safe for use. This length of time varies depending on materials and product, how it is used, and how it is stored. PGE repellent formulations were prepared from 20 to 40 mL PGE solution, 10–40 mL sodium lauryl ether sulfate as an emulsifier, 0–0.5 g (with/without) sodium benzoate as a preservative, and 10 mL regular mineral water according to the study of Rastogi et al. (2022) [11]. However, we modified the observation period and kept the PGE formulation for 1–8 weeks at room temperature to observe any signs of damage or fungal growth. Here, the prepared formulation was divided into two parts, where sodium benzoate was added in one part, and the other contained no preservatives. Both formulation's shelf life was studied after 8 weeks.

# 2.6. Mosquito repellence activity test

# 2.6.1. Cone bioassays test

Following the World Health Organization guideline [50], four cones were prepared for the cone bioassays test (See Fig. 4). The test setup was composed of transparent cones (diameter 13.5 cm, height: 18 cm) placed over a study sample fabric ( $15 \times 15$  cm) at a 60° inclined test apparatus. An untreated cotton fabric was used to block the cone's narrow rim, via which ten mosquitoes were put into the cone. The loaded mosquitoes were in contact with the study fabrics for 3 min. Then, tested mosquitoes were taken out of the cones and placed in a small insecticide-free air-based cage that works as an aspirator system with access to the sugar solution at  $25 \pm 2$  °C with 50–70% RH for 24 h observation. The percentage of mosquito mortalities/knockdowns was determined after 60 min and 24 h of observation data of the alive and dead mosquitoes using the below formula [8,51].

Percentages of Mosquito Mortality/knockdown = 
$$\frac{Number of mosquitos knocked over/dead}{Total number of mosquitos introduce} \times 100$$

For each fabric sample (A, B, C, and D), the trials were performed three times to observe the mosquito's living conditions. Here, the mosquitoes that cannot stand or fly coordinately, hardly move their legs or wings, and fall immediately were defined as moribund. The immobile mosquitoes that did not show a sign of life after 60 min were expressed as knockdown and were named dead during the evaluation period [49].

## 2.6.2. Cage test for mosquito repellency

A  $50 \times 30 \times 40$  cm transparent glass cage (See Fig. 5) was modified from the box idea of previous studies by Mia et al. (2020) [17], Halbkat et al. (2019) [52], and Teli & Chavan et al. (2017) [16]. We determined the mosquito-repelling performance of PGE-treated sample fabric without using a human touch, which was in contrast to previous studies. In the modified cage, the temperature was maintained similar to room temperature at  $25 \pm 2$  °C, with a relative humidity range of 60–70% and the experiment was performed during daylight time. The test cage was designed by attaching the control sample on the wall of one side, while the other side used a PGE sample, and the top of the cage was left blank. Here, the control fabric sample means a fabric without any treatment. The bottom of the cage was half-covered with a PGE-treated fabric sample and half-covered with a control or untreated one. The rest of the two sides were covered, one with the PGE-treated sample and the other with an untreated sample. Twenty-five mosquitoes were released into the cage and left for two to 3 min to settle. Each test was replicated three times and each time the mosquito repellency of fabrics was observed after 5, 30, and 60-min intervals. After calculating the average of how many mosquitoes settled/landed on the test samples, the repellency percentage was measured according to the study of Rastogi et al. (2022) [11]. The second phase of the investigation was conducted 15 days after the first investigation period.



Fig. 4. Schematic diagram of cone bioassays test.



Fig. 5. Schematic diagram of cage bioassays test.

Mosquito repellency 
$$\% = \frac{T - D}{T} \times 100$$

Here, the total exposed specimen (T), the number of mosquitos that landed or settled on treated (D).

### 2.7. Effect of washing of PGE treated fabrics

The effect of washing was evaluated in two different ways. In the first type of investigation, the durability of the mosquito finish materials on knitted fabric samples was investigated after 1, 3, and 5 consecutive washes. The PGE-treated samples were washed at 40 °C for 30 min, according to ISO 105-C06 (2010) [53], where a non-phosphate washing detergent (3.5 g/L) was used. After washing, the fabrics were rinsed and dried in laboratory conditions [51]. Then, the investigation of the washing cycle's impact on the number of mosquito mortalities was conducted twice for each fabric following the cone bioassay test for the washed PGE-treated samples [49]. The second way of assessing washing effectiveness was by analyzing the weight of PGE-treated or dyed fabric samples. Here, the washed fabric weight was compared to PGE-treated fabric and also with the untreated or control fabric weight. For better accuracy of weight measurement, a 7 × 2 cm length and width fabric sample was cut from A, B, C, and D PGE-treated samples, including the control one .

#### 2.8. Fabric color fastness

#### 2.8.1. Color fastness to wash

Color fastness to washing means the durability of finishing materials or dyes used in textile finishing when a textile specimen is agitated under a certain time and temperature in a soap solution with one or two specified adjacent fabrics. The color fastness to washing for all PGE finished specimens was measured following the ISO 105C06 test method. The examiner then used the grey scale to assess the degree of color change and the staining of the adjacent fabric [53].

#### 2.8.2. Color fastness to rubbing

Color fastness to rubbing/crocking has been developed to assess how well a fabric will resist certain crocking strains. In this experiment, the dyed fabrics' dry and wet rubbing fastness were tested according to the ISO 105 X12:2016 test method. Following the test method, fabric specimens were rubbed against a white fabric to see how much color rubbed off on the test cloth. The examiner then used the grey scale to assess the degree of color change [54].

#### 2.8.3. Color fastness to light

Light fastness, or color fastness to light, is the resistance of finishing materials or dyes to fade or color change due to exposure to sunlight or an artificial light source. The light fastness was determined using artificial illumination, D65, with a Xenon arc light source by following the tested method ISO 105-B02 [55].

## 2.9. Determination of add-on percentage

The add-on % of the PGE-treated fabrics was determined using the gravimetric principle [56]:

Add on 
$$\% = \frac{m_2 - m_1}{m_1} \times 100$$

Here,  $m_1$  and  $m_2$  are referred to as the oven-dried weight of the control and PGE-treated fabric samples, respectively. The reported results are an average of five tests in each case.

#### 2.10. Statistical analysis

One way analysis of variance (ANOVA) was performed on cone bioassay and cage test data of the experiment. A p-value of 0.05 was taken into consideration, and a value of 0.05 or above indicates that there were no significant differences among the data from the four group of finished fabrics.

# 3. Result and discussions

## 3.1. Preparation of formulations and evaluation of their shelf life

There was no sign of fungal growth or deterioration in either case (with or without  $C_7H_5NaO_2$ ), even after eight weeks of storage at room temperature. This may be possible because garlic has long been considered a powerful natural antifungal due to its antifungal sulfur compounds [57]. Similarly, peppermint is known for several medicinal qualities and has been proven to have antifungal properties [36]. Therefore, mixing these two compounds made a strong fungal-resistant solution, which helped the PGE-treated fabric to prevent any fungal growth. Hence, this formulation can be used for apparel and kept for long periods.

## 3.2. Investigation of fastness, add on%, fabric appearance

The fabric's rubbing, wash, and light fastness depend on the amount of unfixed dye, the molecular weight of the dye, the combination of the dye and the fiber, and the uniformity of the dye penetration [58]. As shown in Table 2, sample A has the highest wash, rubbing, and light fastness; in contrast, sample D has the lowest. This may be because increasing the dye's concentration increased the dye molecules' size and more add-on of dyes on fabric surface, reducing dye penetration into the fabric. Moreover, we did not use any fixing agent to fix the floating dyes on the fabric; therefore, samples B, C, and D dyed with high concentration showed poor rubbing and wash fastness. In addition, the binding force between dye and fabric is easily destroyed due to the presence of moisture, so wet rubbing fastness is lower than dry rubbing fastness for all tested samples. Similarly, dye particles that are properly fixed to the fiber show good resistance to light, whereas dyes unfixed to the fiber show poor resistance [59]. Due to low dye concentration, sample A had less unfixed dye, which made it capable of showing good light fastness compared to other samples. Overall, satisfactory fastness ratings such as washing fastness (4/5-3/4), dry rubbing fastness (4/5-4), wet rubbing fastness (4-3/4), and light fastness (8-6/7) were observed for dyed samples. The excellent color fastness of the treated samples confirms the successful bonding of PGE-repellent dye to cotton fabric. For determining the add-on %, a 7  $\times$  2 cm length and width fabric sample was cut from all treated samples, including the controlled one, where the controlled sample weight was found to be 0.3625 gm.

#### 3.3. Cone bioassays test

Cone bioassay plays an integral role in evaluating the efficacy of long-lasting insecticidal nets and insecticide fabric product evaluation. And the cone setup angles under the different settings are important to evaluate the test result [60]. Following WHO standards, the toxicity of the formulation of PGE-treated fabric samples was evaluated based on the knockdown and mortality of mosquitoes reported after 60 min and 24-h intervals, respectively. The findings of knockdown and mortality rates are tabulated in Table 3.

After the first observations (60 min), no knockdown and moribund appeared for sample A; in contrast, an insignificant percentage of knockdowns and moribund was observed for sample B, C, and D, 3.33%, 6.67%, and 13.33%, respectively. However, a one-way ANOVA confirms that the number of mosquitoes knock down by different samples in not statistically significant (F (3, 8) = 3.888, p = 0.055).

After 24 h, the mortality rate increased gradually with increasing PGE concentration; mortality for samples A, B, C, and D was found to be 6.67%, 16.67%, 50.00%, and 76.67%, respectively. Furthermore, a one-way ANOVA also confirms the statistically significance (F

| Effect of PGE sample's different fastness properties with their color appearance. |         |         |               |         |          |               |       |  |  |  |
|---|---------|---------|---------------|---------|----------|---------------|-------|--|--|--|
| Fabric code   | Conc. % | Add on% | Wash fastness | Rubbing | fastness | Lightfastness | Shade |  |  |  |
|   |         |         |               | Dry     | Wet      |               |       |  |  |  |
| Sample A  | 5       | 6.73    | 4/5           | 4/5     | 4        | 8             |       |  |  |  |
| Sample B  | 15      | 10.53   | 4             | 4       | 4        | 7–8           |       |  |  |  |
| Sample C  | 25      | 14.57   | 4             | 4       | 3/4      | 7             |       |  |  |  |
| Sample D  | 35      | 18.65   | 3/4           | 4       | 3/4      | 6–7           |       |  |  |  |

## Table 2

\_ \_ \_ ala'a diffa

#### Table 3

Mosquitos mortality percentage - cone bioassays test.

| after 60 min of observations |           |                    |        |         |               |  |  |  |  |  |
|------------------------------|-----------|--------------------|--------|---------|---------------|--|--|--|--|--|
| Fabric Code                  | Number of | moribunds or Knocl | xdowns | Average | Mortality (%) |  |  |  |  |  |
|                              | I         | п                  | III    |         |               |  |  |  |  |  |
| Sample A                     | 0         | 0                  | 0      | 0.00    | 0.00          |  |  |  |  |  |
| Sample B                     | 1         | 0                  | 0      | 0.33    | 3.33          |  |  |  |  |  |
| Sample C                     | 1         | 1                  | 0      | 0.66    | 6.67          |  |  |  |  |  |
| Sample D                     | 2         | 1                  | 1      | 1.33    | 13.33         |  |  |  |  |  |
| after 24 h of observati      | ions      | _                  |        |         |               |  |  |  |  |  |
| Sample A                     | 0         | 1                  | 1      | 0.67    | 6.67          |  |  |  |  |  |
| Sample B                     | 2         | 1                  | 2      | 1.67    | 16.67         |  |  |  |  |  |
| Sample C                     | 6         | 4                  | 5      | 5.00    | 50.00         |  |  |  |  |  |
| Sample D                     | 7         | 7                  | 9      | 7.67    | 76.67         |  |  |  |  |  |

Note: I, II, and III represent the number of tests conducted for each sample.

(3, 8) = 41.000, p = 0.000) of the data. Moreover, the nozzle was set at  $60^\circ$ , which is probably one of the main reasons for showing a good mortality rate [59]. Overall, it can be assumed that PGE-treated fabric became more effective in knockdown and moribund the mosquitoes after a sufficient period.

# 3.4. Cage test

The cage test is a fast and effective approach to assess the viability of repelling substances against mosquitoes. This test is designed to observe mosquitoes landing on the untreated and treated fabric, which will provide the real situation of the probing and biting of the mosquito to the human. Thus, it helps to notice the mosquito's behavior toward the treated samples [61]. This experiment recorded three intervals of 5, 30, and 60 min to better understand the PGE-treated fabric's performance.

From Table 4, it appeared that the newly treated fabric showed decent mosquito repellency, which varied in the range of 49.3-85.6% among treated samples A to D within 60 min observation period. Among them, sample D (35% concentration PGE) showed the highest (100%, 93.3%, and 85.6%), and sample A (5% concentration PGE) showed the lowest (84%, 73.3%, and 49.3%) mosquito repellency performance among all observation intervals. This happened because peppermint leaves are widely used for their anesthetic, antimicrobial, antifungal, antihelmintic, and antioxidant properties [62]. On the other hand, crushing the garlic bulbs releases allicin, which is thought to play an important role in garlic's antibacterial and antifungal properties [63]. It can be assumed that mixing peppermint and garlic made a strong antiseptic substance that effectively repels mosquitos. Additionally, increasing the PGE concentration gradually increased the mosquito repellency; therefore, sample D showed the highest performance. Furthermore, a one-way ANOVA was conducted on the data gathered for each interval. In all three-time intervals (5, 30 and 60 min), statistically significant difference (p = 0.001, p = 0.000, p = 000 for 5, 30, and 60 min data points, respectively) was found for the mosquito repellency performance among four fabric samples.

Furthermore, after 15 days of the first investigations, the trial was conducted again to observe the treated fabric's repellency performance and optimal results was found as reported in Table 4. Within a 60-min observation period, the 15-day-old treated specimens displayed a mosquito repellency ranging from 41.3 to 84% among treated samples A to D. Among them, sample D (35%

## Table 4

Assessment of percentage of mosquito repellency in a Cage test method.

| Observation (Days)                                | No. of investigations | After fab | ric developm | ent  | After 15 days |      |      |
|---|-----------------------|-----------|--------------|------|---------------|------|------|
| Observation (Minutes)                             |                       | 5         | 30           | 60   | 5             | 30   | 60   |
| No. of mosquitoes escaped and settled on Sample A | I                     | 5         | 7            | 14   | 5             | 9    | 14   |
|   | II                    | 3         | 7            | 13   | 4             | 7    | 16   |
|   | III                   | 4         | 6            | 11   | 5             | 8    | 14   |
| Average repellency (%)                            |                       | 84.0      | 73.3         | 49.3 | 81.3          | 68.0 | 41.3 |
| No. of mosquitoes escaped and settled on Sample B | I                     | 2         | 5            | 7    | 3             | 8    | 12   |
|   | II                    | 4         | 5            | 10   | 3             | 8    | 13   |
|   | III                   | 3         | 5            | 9    | 6             | 10   | 11   |
| Average repellency (%)                            |                       | 88.0      | 80.0         | 65.3 | 84.0          | 65.3 | 52.0 |
| No. of mosquitoes escaped and settled on Sample C | I                     | 1         | 4            | 6    | 1             | 6    | 7    |
|   | II                    | 2         | 4            | 5    | 3             | 5    | 7    |
|   | III                   | 2         | 5            | 5    | 4             | 7    | 6    |
| Average repellency (%)                            |                       | 93.3      | 82.7         | 78.6 | 89.3          | 76.0 | 73.3 |
| No. of mosquitoes escaped and settled on Sample D | Ι                     | 0         | 2            | 5    | 0             | 1    | 7    |
|   | II                    | 0         | 1            | 2    | 0             | 1    | 2    |
|   | III                   | 0         | 2            | 4    | 0             | 3    | 3    |
| Average repellency (%)                            |                       | 100       | 93.3         | 85.6 | 100           | 93.3 | 84.0 |

Note: for each investigation, 25 numbers of mosquitos were initially introduced.

 Table 5

 Effect of washing the PGE-treated fabric samples.

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| No. of<br>Wash | Sample A               |                            |                      | Sample B               |                            |                      | Sample C               |                            |                      | Sample D               |                            |                      |
|----------------|------------------------|----------------------------|----------------------|------------------------|----------------------------|----------------------|------------------------|----------------------------|----------------------|------------------------|----------------------------|----------------------|
|                | Initial<br>Weight (gm) | After wash<br>Weight (gm)  | Weight Loss<br>(%)   | Initial<br>Weight (gm) | After wash<br>Weight (gm)  | Weight Loss<br>(%)   | Initial<br>Weight (gm) | After wash<br>Weight (gm)  | Weight Loss<br>(%)   | Initial<br>Weight (gm) | After wash<br>Weight (gm)  | Weight Loss<br>(%)   |
| 1<br>3<br>5    | 0.3869                 | 0.3807<br>0.3714<br>0.3636 | 0.62<br>1.55<br>2.33 | 0.4007                 | 0.3943<br>0.3846<br>0.3766 | 0.64<br>1.61<br>2.41 | 0.4153                 | 0.4086<br>0.3987<br>0.3904 | 0.67<br>1.66<br>2.49 | 0.4301                 | 0.4232<br>0.4129<br>0.4043 | 0.69<br>1.72<br>2.58 |

#### Table 6

Effect of washing cycles on the number of mosquito mortalities after 24 h.

| Number of washes | Sample A |    |       | Samp | Sample B |       | Sample C |   |       | Sample D |    |       |
|------------------|----------|----|-------|------|----------|-------|----------|---|-------|----------|----|-------|
|                  | I        | II | Aver. | Ι    | II       | Aver. | I        | п | Aver. | I        | II | Aver. |
| 0                | 1        | 1  | 1.00  | 2    | 3        | 2.50  | 5        | 6 | 5.50  | 9        | 7  | 8.00  |
| 1                | 0        | 0  | 0.00  | 1    | 1        | 1.00  | 3        | 3 | 3.00  | 6        | 6  | 6.00  |
| 3                | 1        | 0  | 0.50  | 0    | 1        | 0.50  | 2        | 1 | 1.50  | 5        | 4  | 4.50  |
| 5                | 0        | 0  | 0.00  | 0    | 0        | 0.00  | 0        | 1 | 0.50  | 3        | 2  | 2.50  |

concentration PGE) had the best performance in repelling mosquitoes (100%, 93.3%, and 84%) while sample A (5% concentration PGE) demonstrated the worst performance (81.3%, 68%, and 41.3%) throughout all observation periods. Additionally, during each of the three-time intervals (5, 30 and 60 min), a statistically significant difference (p = 0.006, p = 0.000, and p = 000 for the relevant 5, 30, and 60 min data points) was discovered in the effectiveness of the four fabrics' ability to repel mosquitoes. This outcome closely resembles that the newly treated fabric specimens after 15 days aging did not affect the mosquito repellency of the treated fabric.

#### 3.5. Analysis of PGE-treated fabric after consecutive washing cycles

The wash-off test was used to understand the impact of washing cycles on the PGE-treated samples. The fabric was washed one, three, and five times and the data was analyzed. After analyzing Table 5, we found that each fabric's weight reduced gradually with the number of increasing washing cycle (Humidity and drying process).

As we did not use any particular binding or fixing agent except regular dyeing salt and soda during dyeing, this could be one of the probable reasons for the removal of PGE-repellent material. However, after the fifth wash, the weight of sample A almost closer to the controlled fabric (0.3625 gm); on the other hand, samples C and D's weight resembles samples B and C's primary condition. This result also indicates that highly concentrated PGE-treated fabric is still effective after the fifth wash. It can be seen from Table 6 that the mortality rate of PGE-treated fabric decreased significantly for all fabric samples with increasing the washing cycles.

Samples D and C had a decent morality rate than the other two samples after the first wash. However, a steep decrement in mosquito mortality numbers was found after the third and fifth washes. Especially, samples A and B showed almost zero performance on mosquito mortalities. It was apparent as PGE repellent material was almost washed off after the third and fifth washes. From Tables 6 and it can be seen that Mosquito knockdown or killed ability decreased proportionally to the weight loss of the PGE-treated fabric. However, the ability of mosquito repellency of the treated fabric will remain active when the fabric contains PGE materials on its surface in our hypothesis.

## 4. Conclusion and future work

This research study aimed to examine the different characteristics of mosquito-repellent knitted fabric designed using a plant-based extract as a natural dye synthesized from peppermint leaves and garlic. An in-depth description of the experimental procedure was portrayed, from raw material collections to testing the finished fabrics. Finally, the effect of mosquito repellency on PGE-treated fabric was analyzed.

Mixing garlic and peppermint compounds made a strong fungal-resistant material which helped PGE-treated fabric prevent any fungal growth even after eight weeks at room temperature.

Overall, all the fabrics showed satisfactory fastness ratings such as washing fastness (4/5–3/4), dry rubbing fastness (4/5–4), wet rubbing fastness (4-3/4), and light fastness (8-6/7), among them sample A had the highest rating performance. Following WHO standards, a cage and a cone bioassays test were performed. At the time of the cone test, no knockdown and moribund appeared for sample A after the first hour of observations. In contrast, a minor percentage of knockdowns and moribund was observed for samples B, C, and D. However, after 24 h, the mortality rate increased gradually with increasing the PGE concentration, and mortality for sample A found to be the lowest (6.67%), and sample D found the highest (78.67%).

On the other hand, the study took three observation intervals of 5, 30, and 60 min at the time of the cage test. The treated fabric showed decent mosquito repellency, ranging between 49.3 and 85.6% among treated samples A to D.

Overall, the studied PGE-treated fabric would have a great potential for repelling mosquitoes without creating cytotoxicity to the human body like synthetic repellents; however, more research needs to be done to increase the be durability of PGE-treated fabric's repellent materials. This creates a next massive challenge for us to work with this gap by using an effective natural binder or binding agent to make the plant-based finished materials more durable after consecutive laundering.

In this work, the proof of concept for employing a peppermint-garlic (3:1) mixture as a viable component for knit fabric substrate that can deter mosquitoes was investigated. To find the ideal formula, other peppermint and garlic combo ratios might be investigated further. Also, it would be intriguing to investigate how various fabric kinds (woven and knit) with varying fiber blend ratios interact with PGE solution. In order to determine whether the PGE solution is bonded in the fabric and fiber scale, the chemical analysis of the treated sample can also be performed. Also, in future studies, it may be worthwhile to investigate the color strength of fabrics with PGE finishes in order to obtain a quantitative color value for the PGE finish in fabrics.

#### Declarations

#### Author contribution statement

Abdullah Al Parvez: Conceived and designed the experiments; Wrote the paper.Md. Jakir Hossain, Md. Saiful Hoque: Analyzed and interpreted the data; Wrote the paper.Md. Zabed Hossain, Mohammad Sazzad Hossain Sohan, Fariha Hoque, Md. Habibul Ahsan: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

#### Data availability statement

Data included in article/supp. material/referenced in article.

### Additional information

No additional information is available for this paper.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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