Development of insect repellent textiles via treating with microcapsules containing *Citrus aurantium* L. peel essential oil

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ABSTRACT – REZUMAT

Development of insect repellent textiles via treating with microcapsules containing *Citrus aurantium* L. peel essential oil

This study aimed to extract Citrus aurantium L. (bitter orange) peel essential oil (CPEO) and test its repellent effect against Culex mosquitoes by incorporating it into textiles in the form of microcapsules. CPEO was obtained from dried citrus peel using the hydrodistillation method with the Clevenger apparatus. Microcapsules containing the essential oil were formed using the complex coacervation method. The volatile compounds of the microcapsules containing CPEO were characterized by gas chromatography-mass spectrometry (GC-MS). Microcapsules with a spherical and uniform shape, as well as appropriate diameter distributions, were observed in optical microscope images and then applied to textiles. The cotton, polyester, and acrylic fabrics were treated with microcapsules using an exhausting method. Scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX) and Fourier transform infrared (FTIR) spectrophotometry analysis were conducted to characterize the fabrics. Finally, an excito chamber test unit was fabricated to evaluate the insect-repellent effects of fabrics against Culex mosquitoes. This study revealed that the fabrics functionalized with CPEO microcapsules exhibit significant repellent effects against Culex mosquitoes.

Keywords: *Citrus aurantium L., complex coacervation, Culex, insect repellent, microcapsules, textiles*

Dezvoltarea de materiale textile insectifuge prin tratarea acestora cu microcapsule care conțin ulei esențial din coaja *Citrus aurantium L.*

Scopul acestui studiu a fost de a extrage uleiul esențial din coaja Citrus aurantium L. (portocală amară) (CPEO) și de a testa efectul său insectifug împotriva țânțarilor Culex, prin încorporarea acestuia în materiale textile sub formă de microcapsule. CPEO a fost obținut din coaja uscată de citrice prin metoda hidrodistilării cu aparatul Clevenger. Microcapsulele care conțin ulei esențial au fost formate prin metoda coacervării complexe. Compușii volatili ai microcapsulelor care conțin CPEO au fost caracterizați prin cromatografie de gaze-spectrometrie de masă (GC-MS). Microcapsulele cu o formă sferică și uniformă, precum și distribuții adecvate ale diametrului, au fost observate în imagini de microscop optic și apoi aplicate pe materialele textile. Materialele textile din bumbac, poliester și cele acrilice au fost tratate cu microcapsule utilizând o metodă de epuizare. Pentru caracterizarea materialelor textile au fost efectuate analize de microscopie electronică de baleiaj cu dispersie de energie (SEM-EDX) și spectrofotometrie în infraroșu cu transformată Fourier (FTIR). În cele din urmă, a fost fabricată o unitate de testare cu cameră excito pentru a evalua efectele repelente ale materialelor textile împotriva țânțarilor Culex. Acest studiu a arătat că materialele textile funcționalizate cu microcapsule CPEO prezintă proprietăți insectifuge semnificative împotriva țânțarilor Culex.

Cuvinte-cheie*: Citrus aurantium L., coacervare complexă, Culex, insectifug, microcapsule, textile*

INTRODUCTION

Insects are among the deadliest creatures in the world because they transmit fatal diseases such as plague, malaria, cholera, fever, tularemia, typhus, and Crimean-Congo hemorrhagic fever [1]. Many tropical diseases, including malaria, dengue, yellow fever, and filariasis result from the bites of infected female mosquitoes of the genera *Aedes Meigen*, *Anopheles Meigen, Culex L.*, and *Haemagogus L.* bloodsuckers [2, 3]. Malaria is specifically caused by transmitting the *Plasmodium* parasite, a single-celled organism, to humans by *Anopheles* mosquitoes [4]. In addition, insects and pests cause fear and discomfort in humans. Many synthetic and natural substances are used to protect against insects. Synthetic substances typically include pesticides (insecticides), naphthalene, boric acid, and fumigants. However, fumigants and other pesticides can cause health problems such as headaches, nausea, and respiratory issues. Other synthetic insect repellents include N,N-diethyl-meta-toluamide (DEET), picaridin, and permethrin [5]. Since the body absorbs these synthetic substances and can remain under the skin, they may cause health problems when used over long periods [6]. Recent increases in these health problems have led people to seek natural alternatives to synthetic and hazardous substances. Citronella, neem, and lemon eucalyptus are among the most effective natural insect-repellent agents [5].

Nowadays, the importance of multidisciplinary studies is increasing. Research that combines fields such as food science, chemistry, pharmacy, and textile science is becoming more common. While chemists, food scientists, and pharmaceutical researchers study synthetic and natural compounds, textile scientists focus on methods for producing and analysing insect-repellent textile materials containing these compounds. To date, various methods have been utilized in the production of insect repellent textiles including electrospinning techniques, microcapsule applications, and cyclodextrin applications [5, 7]. Microencapsulation is the most preferred method [8] due to its advantages of ease of application, cost-effectiveness, and suitability for scale-up [9–12]. Additionally, microencapsulation ensures the long-term preservation of the encapsulated agent and maintains the effectiveness of essential oil components over time [13].

The effectiveness of insect-repellent textiles can be tested by various methods. The most commonly used techniques are cage test, cone test, and excito chamber test [5, 14]. In the cage test, which evaluates repellent efficiency against mosquitoes, the cage sizes should be between 35–40 cm according to the World Health Organization (WHO) [15]. Some studies have reported modifications to cage sizes. Bano [16] used a cage with dimensions of 18×18×18 cm^3 whereas Phasomkusolsil and Soonwera [17] used a cubic cage with a unit length of 30 cm. Anitha et al. [18] chose a 32 cm for their cubic cages. Larger cage sizes were tested by Chang et al., Ffei and Xin, and Vigneshkumar and Vijay Kumar Vediappan [19–21]. The cage test is designed to observe the landing of mosquitoes on treated and untreated fabrics. Logan et al. [22] used 50 female mosquitoes in the cage test in their study. The test chamber was 50×50×50 cm^3. In the test method, mosquito-repellent compounds were applied to the forearm from the elbow to the wrist of volunteer subjects. The control arm was placed in a cage, and landings were recorded for 3 minutes while escapes were observed.

In the cone test, parts of human parts are not used as bait for mosquitoes. Instead, artificial or animal blood is used to attract them. A cone is placed over the treated fabric, and mosquitoes are introduced into this cone and exposed to the treated fabric for 3 minutes. The behaviour of the mosquitoes is then observed for an additional 3 minutes [14].

In the excito chamber test method, there are two chambers connected by a tunnel referred to as the exposure chamber and the escape chamber. The escape behaviour of mosquitoes from one chamber to another is examined under exposure to the repellent compound [23].

To date, various studies have been conducted to prevent many mosquito-borne diseases, using both synthetic and plant-based agents. In N'Guessan et al.'s study, DEET (N, N-diethyl-meta-toluamide), a chemical repellent was microencapsulated. It was observed that the encapsulated DEET was released from the microcapsules slowly and was effective in killing the mosquitoes in the environment for at least 6 hours. Additionally, the study demonstrated the applicability of these microcapsules on clothing and bedding materials [24]. Since the chemical mosquito repellents can be harmful to human skin and health, the use of natural agents has gained importance. Although the natural agents do not have the direct killing effect as synthetic repellents, their repellent efficacy has been demonstrated by several studies in the literature [25, 26].

In the study conducted by Anitha et al., 100% polyester fabrics were treated with microcapsules containing lemon oil, and their mosquito-repellent effect was evaluated. The tests demonstrated the microencapsulated polyester fabric containing lemon oil exhibited a high level of mosquito-repellent effectiveness [18]. In 2017, Showler examined the repellency of various plant-based substances, including *Allium* (garlic), *Azadirachta* (neem), *Chrysanthemum* (pyrethrum), *Cinnamomum* (cinnamon), *Cymbopogon* (lemongrass), *Derris* (rotenone), *Eucalyptus*, *Festuca* (tall fescue), *Melaleuca* (tea tree), *Melinis* (molasses grass), *Mentha* (mint), *Nepeta* (catnip), *Nicotiana* (tobacco), *Pelargonium* (geranium), *Pogostemon* (patchouli), *Ricinus* (castor bean), *Rosa* (rose), *Syzygium* (clove), *Vitex* (monk's pepper), and *Zyloxanthum* (Japanese pepper), against horn flies and horse flies which are blood-feeding ectoparasites of wild and domesticated animals [27]. Although plant oils and bioactive compounds are known for their repellent effects, the use of chemicals remains more effective and permanent in some applications [27].

In another study, the microcapsules containing limonene and permethrin with ethyl cellulose shells were produced and applied to cotton fabrics by padding, and their mosquito-repellent effect was tested. The study reported death rates of *Culex* mosquitoes as 41% for limonene and 54% for permethrin. Additionally, it was found that the treated fabrics maintained their repellent effect even after 20 washing cycles [28]. Saeidi et al. investigated the fly-repellent effect of essential oils extracted from the peels of *Citrus reticulata Blanco* (tangerine), *Citrus limon* L. (lemon), and *Citrus aurantium* L. (bitter orange). They used an olfactometer to evaluate the repellency of these essential oils. The results indicated that all tested Citrus peel essential oils had a strong fly-killing and repellent effect [29]. Prakash et al. applied extracts of *Citrus sinensis* (sweet orange) and *Citrus aurantifolia* (lime) extracts to cotton nets to enhance their mosquito-repellent effect. They conducted the cage test, modified excito chamber test, antibacterial test, and washing resistance tests on the nets. The results showed that the extracts had an antibacterial effect against *Escherichia coli* and *Staphylococcus aureus,* and the nets exhibited a repellent effect after several washings [30].

Flies and insects in the environment are not only disturbing but also can cause allergic reactions upon contact with the skin. Most natural fly and insect repellents are available in the form of essential oils. However, the volatile nature of these oils results in a temporary effect that fades quickly. To achieve a long-term effect, it is necessary to release these compounds at regular or sustained intervals.

Unfortunately, controlled release is challenging with traditional textile finishing methods. Recently, textile researchers have increasingly focused on microencapsulation to extend the effective period and reduce the rapid release of essential oils from fabrics.

This study aimed to produce microcapsules containing *C. aurantium* peel essential oil (CPEO), a natural and aromatic agent, treat three types of fabrics with these microcapsules, and test the insect-repellent effect of treated fabrics using the excito chamber test method. Another objective of the study was to characterize the fabrics treated with these microcapsules. To the best of our knowledge, no other study has conducted repellency tests on fabrics containing CPEO microcapsules using an excito chamber test unit. Additionally, the volatile compounds of CPEO-containing microcapsules were identified for the first time by GC-MS in this study.

MATERIALS

All chemicals used in the study were obtained from Sigma-Aldrich and were used without further purification. The fruits of *Citrus aurantium* L. plant fruits were collected from the Aegean region of Türkiye. The solid phase micro-extraction (SPME) technique was employed to identify the volatile compounds in CPEO-containing microcapsules.

The filter fabric made of 100 % polyester monofilament yarn with 420 mesh, was purchased from a local supplier. The properties of the fabric onto which the microcapsules were transferred are presented in table 1.

During the exhausting process a styrene-acrylic copolymer-type binder, Pigmacolor BSA was used. Distilled water was employed throughout all experiments, and the tests were conducted in triplicate.

The excito chamber test unit consists of two chambers; the exposure chamber and the escape chamber. Both chambers were made of glass and measured 34 cm × 32 cm × 32 cm, as specified in the literature [18]. The upper part of the exposure chamber is covered with a gauze-like fabric to allow the survival of the mosquitoes placed in the chamber as larvae. A hole with a diameter of 9 cm is drilled into the front surface of this chamber to facilitate easy hand access. This hole is kept closed with a dense fabric to prevent the escape of mosquitoes during rearing. Additionally, the two chambers were connected by a tunnel made of acetate sheet, which had a lid that was easy to open and close manually.

METHODS

Citrus aurantium peel essential oil (CPEO) extraction

In this study, the conventional hydrodistillation technique was chosen for the extraction of *C. aurantium* peel essential oil. First, *C. aurantium* fruits collected from the Aegean region were washed, weighed, and manually peeled in the laboratory. The peels were cut into small pieces (approx. 2×2 cm). To determine the maximum oil yield, preliminary trials were conducted at different solid-to-liquid ratios (w/v). Specifically, 100, 120, 150, 200, and 250 g of dried peels were added to 500 ml of distilled water, and the hydrodistillation was carried out using a Clevenger-type apparatus for a minimum of 3 hours, following the method described by Azhdarzadeh and Hojjati [31]. All extracted essential oils were filtered through anhydrous sodium sulfate and stored in amber vials at 4°C for further analysis.

Preparation of microcapsules

The complex coacervation method was employed for the microencapsulation. Gelatin and gum arabic were used as shell materials, while CPEO served as the core material. Initially, a 2% (w/w) gelatin aqueous solution, the carrier polymer solution, was prepared at pH 7 and 40°C. 4 ml of CPEO were dispersed in this aqueous polymer solution. To form an emulsion, 5% Span80 was added to disperse the water-insoluble CPEO droplets in the aqueous gelatin solution. Emulsion formation was achieved using a mechanical stirrer (M Tops Co, MS-3040 model, Seoul, Korea) at a stirring speed of 1500 rpm. Subsequently, a negatively charged gum arabic aqueous polymer solution (20% w/v) at pH 7 and 40°C was added to the emulsion, stabilizing it with the two polymers. While stirring continued, the pH was adjusted to 4 to form a liquid complex coacervate. This adjustment allowed the polymers to form a polymer complex through electrostatic effects. The pH value of the complex coacervation for gelatin-gum arabic polymers was between 4.0 and 4.5, causing the system to separate into two liquid phases: the coacervate (polymer-rich phase) and the equilibrium solution (polymer-poor phase). The system temperature was

Table 1

THE PROPERTIES OF FABRICS USED

Fabric type	Raw material	Mass per unit area (g/m^2)	Structure	Yarn linear density
1	Polyester	110	Woven	8 Tex multifilament
2	Cotton	59	Woven	19.7 Tex
3	Acrylic	195	Weft knitted	66.7 Tex

then reduced to room temperature with an ice bath. A solid shell formed through the gelation of the gelatin in the coacervate at this temperature. To increase the shell's strength, the temperature was lowered further to 5–10°C. The microcapsule shells were hardened by adding a 25% glutaraldehyde solution in water, which cross-linked the gelatin via reactions with amino groups in the gelatin chain. The microcapsules were placed on the filter fabric, washed with dilute isopropyl alcohol (30% v/v) to remove the excess oil, and rinsed three times. The microcapsules were stored as is at +4°C until use. The complex coacervation process parameters, including temperature, pH, stirring speed, and polymer concentration were optimized based on optical microscope analyses.

These parameters resulted in microcapsules with uniform morphology and minimal diameter distribution. Initially, optical microscope images of the microcapsules were captured, and diameters and diameter distributions were measured using ImageJ Measurement and Visualization Software.

The SEM-EDX analysis (LEO 1430 VP Leo Electron Microscopy Ltd., Cambridge, UK) was performed to determine the morphology and elemental composition of the microcapsules.

GC-MS analyses

For GC-MS analyses, the microcapsules were dried to ensure reliable results. The volatile compounds in CPEO were determined using the solid phase microextraction (SPME) technique [33] with a GC-MS (GC 6890, MS 6890N, Agilent Technologies, Wilmington, DE, USA). The HP-5MS column, which is nonpolar (30 m × 0.250 mm id × 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA) was used for the separation of volatile compounds. One gram of CPEO was weighed into a 40 ml SPME vial (Supelco, Bellefonte, USA) and kept in a 40°C water bath (GFL, Model 1103, Burgwedel, Germany) for 20 minutes. Subsequently, the SPME fibre (2 cm to 50/30 lm DVB/Carboxen/PDMS, Supelco, Bellefonte) was inserted into the headspace of the vial and kept in 40°C water bath for an additional 20 minutes before being immediately injected into the device. The flow rate of helium selected as the carrier gas was set at 1.2 ml/min. The temperature program of the GC oven was as follows: 40°C for 5 minutes, then it raised from 40 to 230°C at a rate of 10°C/min and held for 20 min at this oven temperature. The operating conditions of the detector of MS were 280°C capillary direct interface temperature, 70 eV ionization energy, 35–350 amu mass, and 4.45 scans/s scan rate. Identification of volatiles in CPEO was based on mass spectra comparison of unknown compounds with those in the National Institute of Standards and Technology (NIST) and the Wiley Mass Spectral Data Registry, databases [34]. The amounts of volatile compounds are expressed as percentage (%) through peak normalization. The tests were repeated twice. All essential oils were injected twice.

Collection of mosquito larvae/rearing

In this study, *Culex* mosquito larvae were collected along with water from stagnant areas. The larvae were transferred to the laboratory and placed in petri dishes containing the collected water, which was then introduced into the exposure chamber. The gauze-like fabric used as a cover facilitated the air transfer necessary for larvae to undergo their transformation into pupae, a process that took approximately one week. The pupal stage lasted for 3–4 days. Eventually, the pupae matured into adult mosquitoes (teneral stage), which began to fly within the chamber. The adult mosquitoes were fed with a sugar/water solution until the tests were conducted. The stages of rearing are illustrated in figure 1.

Treatment, morphological and structural characterizations of fabrics

Microcapsules produced with optimum process parameters were applied to the fabrics specified in table 1, using the exhaustion method. Fabric samples measuring 5×5 cm^2 were fully immersed in a treatment bath containing Pigmacolor BSA binder (5% v/v) and microcapsules (7.5% w/v). The liquor ratios used were 9:1, 17:1, and 5:1, corresponding to microcapsule concentrations of 1.32, 2.52, and 5.43 g/L for cotton, polyester, and acrylic fabrics, respectively. Each mixture was stirred for 15 minutes at room temperature. Subsequently, the fabrics were removed from the bath, the excess solution was removed, and the fabrics dried at room temperature.

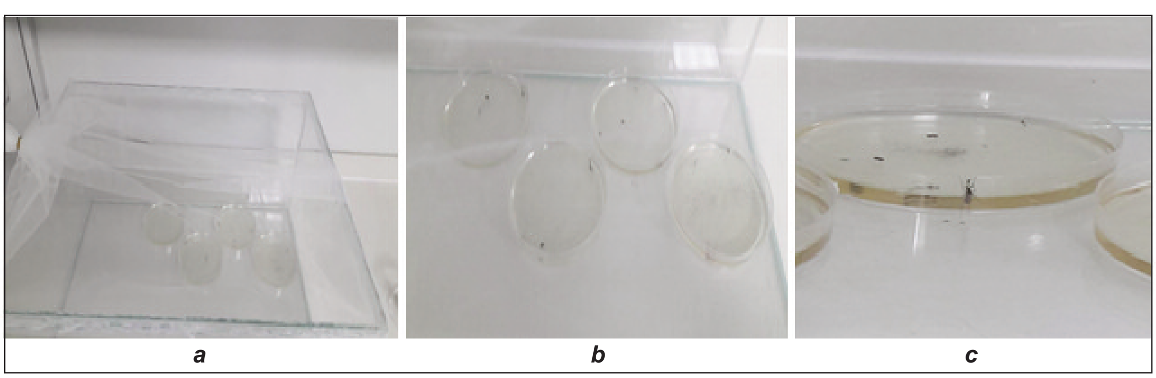

Fig. 1. Mosquitoes: *a* – larval; *b* – pupal; *c* – adult

Following the treatment, the fabrics underwent morphological and elemental analyses using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX) (LEO 1430 VP (Leo Electron Microscopy Ltd., Cambridge, UK)), and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrophotometry (Spectrum Two, Perkin Elmer, USA). SEM-EDX analyses were conducted at an accelerating voltage of 20 kV, with the samples being gold-sputtered before analysis. Both microcapsules and fabrics (untreated and treated) were analysed individually. The ATR-FTIR spectrophotometry, which is suitable for thin surfaces and soft polymers, was employed to record spectra in the range of 4000–400 cm^{-1}, presented as wavenumber versus transmittance (%).

Repellency tests

In this study, the repellent effects of fabrics treated with CPEO microcapsules against mosquitoes were evaluated using the excito chamber test unit, as shown in figure 2.

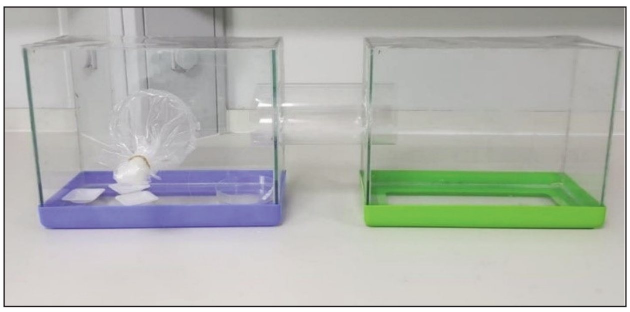

Fig. 2. Excito chamber test unit: exposure chamber (left) and escape chamber (right)

For the excito chamber test of adult *Culex* mosquitoes, initially, 10 mosquitoes were placed in the exposure chamber. Next, 5×5 cm^2 fabric samples were individually introduced into the exposure chamber, while the partition between the chambers was kept closed. Each fabric was placed separately, and light pressure was manually applied (tabbing) to simulate body contact during movement, which facilitated the release of the oil from the microcapsules into the air. The exposure duration was set between 10–12 minutes based on the recommended time in the literature [14] and the retention times of the most abundant volatile compounds in CPEO. After the exposure period, the partition between the chambers was opened manually. The escape behaviours of the mosquitoes to the escape chamber were analysed using the equation provided in reference [32]:

$$\textit{Efficiency of mosquito repellency } (\%) = \\ = \frac{\textit{Number of the mosquitos escaped} + \textit{Number of the mosquitos dead}}{\textit{Number of the mosquitos exposed}} \times 100 \quad (1)$$

The tests were repeated twice. Additionally, a control test was performed with the lid open, where no exposure sample was placed in the exposure chamber.

RESULTS AND DISCUSSION

CPEO hydrodistillation

The conventional hydrodistillation technique was employed for the extraction of *C. aurantium* peel essential oil. The optimum solid/liquid ratio for achieving the highest essential oil yield (%) was determined to be 200 g of dried peels in 500 ml of distilled water, among the tested ratios of 100, 120, 150, 200, and 250 g. The highest yield observed in this study was 1.7 ml/kg which is consistent with the range of 1.2–4.6 ml/kg reported by Bourgou et al. [35] for bitter orange peel essential oils.

Microencapsulation and GC-MS results

The optical microscope and SEM images of the microcapsules produced using the complex coacervation method are shown in figure 3. The microcapsules had spherical structures with an average diameter of 68.5±34.2 μm based on measurements of 50 microcapsules using ImageJ software.

After the microencapsulation of CPEO, the microcapsules were characterized by GC-MS to identify the volatile compounds. The compound names, retention times, and percentages calculated from peak areas normalization are provided in table 2. A total of 44 compounds were identified and quantified. The most abundant volatile and aromatic compound in the encapsulated CPEO was D-limonene, which constituted 39.14% of the total. This finding is consistent with reports from various researchers indicating that D-limonene is responsible for the characteristic citrus aroma in all citrus varieties, often comprising up to 90% of the volatile compounds in bitter orange [39, 40]. Other major volatile compounds identified were p-xylene, linalool, and mentha-1,4,8-triene, with percentages of 5.71%, 3.78%, and 3.74%, respectively. While the volatile profile of CPEO aligns with previous literature, it is important to note that variations in volatile compounds can arise due to climatic conditions, geographical origins, and different oil extraction techniques, even for the same plant species.

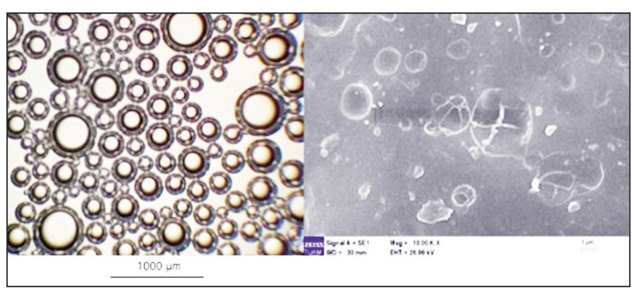

Fig. 3. Optical microscope (X40) and SEM images (X10 K) of microcapsules

D-limonene, the predominant volatile compound in the microcapsules, is known for its insect-repellent properties [41]. This finding supports the observed repellent effects of fabrics treated with D-limonene-rich microcapsules.

Table 2

VOLATILE COMPOUNDS OF CPEO-C MICROCAPSULES			
Compound	**Retention time (min)**	**Area (%)**	
		Mean	**Std. Dev.**
Methyl-d3 1-Dideuterio-2 propenyl Ether	2.963	0.12	0.08
Pyridine	4.007	2.07	1.33
Hexanal	5.286	1.99	1.11
p-Xylene	7.441	5.71	2.86
α-Pinene	7.441	0.45	0.07
Benzaldehyde	8.889	1.17	0.53
Benzoic acid	9.544	2.56	2.25
β- Pinene	9.655	0.88	0.18
m-Cymene	10.173	0.46	0.05
D-Limonene	10.804	39.14	16.50
Hexadecane, 2,6,10,14-tetramethyl-	11.368	0.31	0.17
Linalol oxide	11.732	1.86	0.17
cis-Linalol oxide	12.01	0.94	0.19
Linalool	12.194	3.78	0.05
3-Ethyl-3-methylheptane Heptane, 3-ethyl-3-methyl-	12.297	1.18	0.07
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	12.793	0.88	0.06
L-Camphor	12.971	0.67	0.41
Borneol (1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol)	13.316	0.77	0.02
Terpinen 4-ol	13.485	0.41	0.08
m-Thymol	13.59	0.26	0.03
α-Terpineol	13.691	2.75	1.10
P-Menth-6-en-2,3-diol	13.847	1.69	0.46
cis-Carveol	14.112	1.58	0.52
Nerol	14.227	0.66	0.26
p-Cumic aldehyde	14.44	0.31	0.17
Carvone	14.503	1.73	0.02
trans-Geraniol	14.604	1.11	0.96
Decanedioic acid, didecyl ester	14.956	0.57	0.57
cis-Anethole	15.114	0.53	0.17
p-Thymol	15.283	1.05	0.82
4-carene	15.841	0.93	0.64
3-Methylene-1,5,5 trimethylcyclohexene	15.977	0.61	0.13
α-Cubebene	16.091	0.36	0.28
2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate	16.381	0.22	0.16
3,7-Dimethyl-6-nonen-1-ol	16.716	0.35	0.12
2-ally furan	16.897	0.42	0.01
Caryophyllene	16.996	0.20	0.07
Mentha-1,4,8-triene	17.099	3.74	1.72
α-Caryophyllene	17.437	0.14	0.01
Phenol, 2,5-bis(1,1-dimethylethyl)	18.028	0.29	0.06
1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)	18.204	0.21	0.07
Caryophyllene oxide	19.047	0.53	0.04

SEM-EDX analyses

The SEM-EDX analysis results were examined regarding the detected element average normalized weight percentages. Results are given in table 3. Initially, the microcapsules were analysed, revealing that oxygen was the most prevalent element at approximately 48%, followed by carbon at around 30%. Nitrogen was the least detected element, constituting 21.8% of the microcapsules.

For pure cotton fabrics, approximately 49% consisted of carbon, while about 43% was oxygen, and only 8% was nitrogen. In contrast, cotton fabrics treated with

Table 3

SEM-EDX RESULTS OF MICROCAPSULES AND FABRIC SAMPLES							
Detected element (normalized weight %)	**Microcapsules**	**Fabric samples**					
		Pure cotton	**Treated cotton**	**Pure polyester**	**Treated polyester**	**Pure acrylic**	**Treated acrylic**
Oxygen	47.89	43.27	51.75	51.73	47.13	18.95	16.14
Carbon	30.31	48.71	36.03	48.27	42.55	50.09	53.07
Nitrogen	21.80	8.02	12.22	0.00	10.32	30.96	30.79
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

microcapsules showed increased percentages of oxygen (~52%) and nitrogen (~12%), compared to the untreated cotton. These increases can be attributed to the presence of microcapsules on the fabrics.

SEM-EDX analyses are less effective for detecting elements with low atomic numbers, such as hydrogen. For pure polyester fabrics, the dominant elements were oxygen (~52%) and carbon (~48%). The presence of nitrogen (~10%) in polyester fabrics treated with microcapsules indicates the successful transfer of the microcapsules to the fabric.

In acrylic fabrics, only slight changes were observed. There was approximately a 3% decrease in oxygen and a 3% increase in carbon in the treated acrylic fabrics compared to the pure ones.

Figure 4 displays the SEM images of fabrics treated with microcapsules, illustrating the presence of microcapsules on the fabrics.

FTIR results

Figure 5 presents the FTIR spectrum of the dry microcapsules. The peaks seen in the 1500–400 cm^{-1} fingerprint region correspond to those reported in the literature on pure gelatin and pure gum arabic [42, 43], which are used as shell material. Broadband between 3600–3100 cm^{-1} was formed by -OH stretching of alcohols in the structure. The peak observed at 1722 cm^{-1} is attributed to the C=O stretching [44, 45]. The peak at 1631 cm^{-1} belongs to NH stretching and the presence of amide I [42, 43]. Bending vibrations in NH and CN groups of gelatin (amide II and amide III) cause the peaks at 1537 and 1247 cm^{-1}, respectively [42]. CH bending vibrations in gum arabic cause a peak at 1453 cm^{-1} [43]. Symmetric and asymmetric bending vibrations of both gum arabic and gelatin polymers create a peak at 1380 cm^{-1} [42–44].

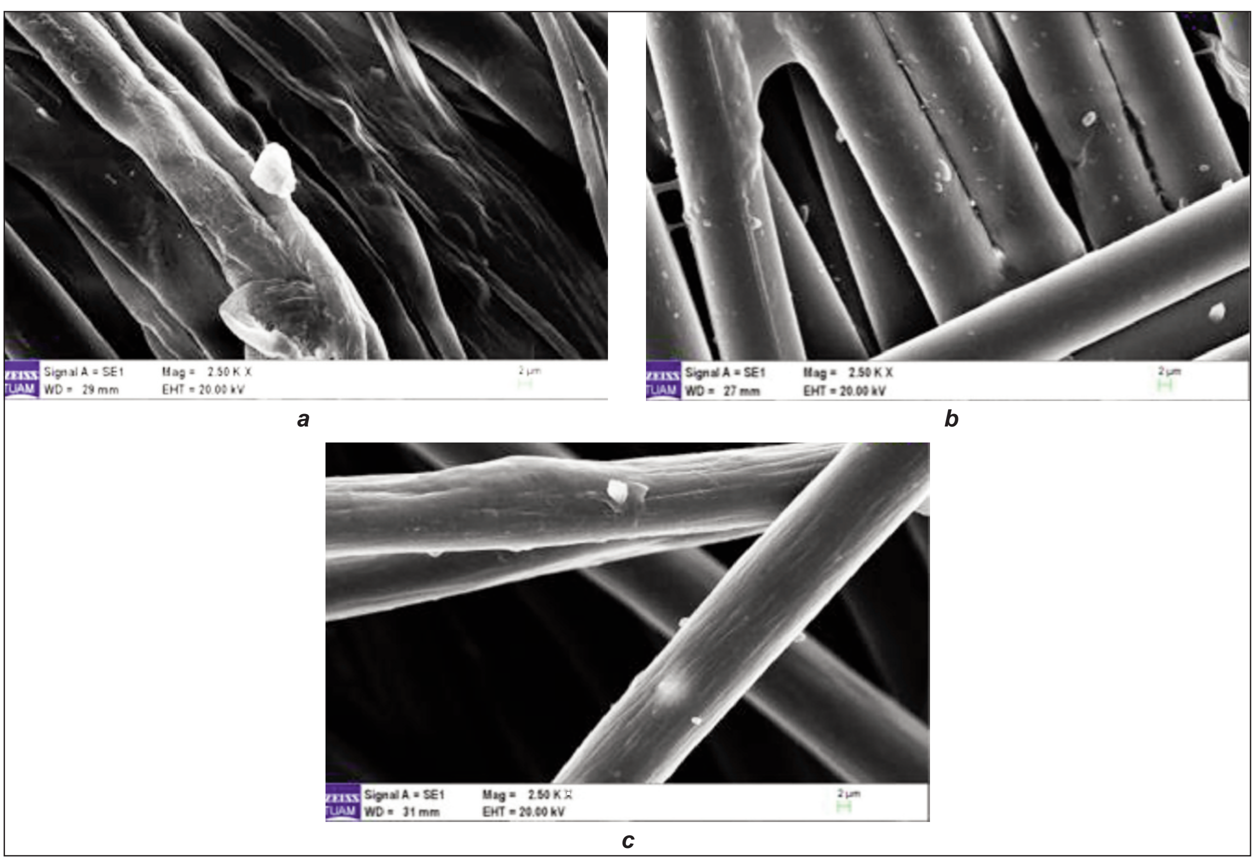

Fig. 4. The SEM images of: *a* – cotton; *b* – polyester; *c* – acrylic fabrics treated with microcapsules (X2.50 K)

As seen from the GC-MS characterization, the main component of the CPEO was D-limonene, whose chemical structure is given in figure 5. The methyl groups of D-limonene can be seen in the image. The peaks observed between 2941–2854 cm^{-1} and 1453–1339 cm^{-1} are attributed to methylene bond vibrations (C-H) [45]. The intense peak at 2924 cm^{-1} is particularly indicative of essential oils [45]. Moreover, peaks at 1722, 1537, and 1408 cm^{-1} are created by the C=C stretching of the aromatic ring [46]. These findings confirm the successful encapsulation of CPEO.

The FTIR spectra of pure and treated 100% cotton fabrics are given in figure 6. The FTIR spectrum of the pure cotton fabric aligns with literature values in the 1500–450 cm^{-1} wavelength region [47, 48]. A low-intensity peak was observed at 1734 cm^{-1} in the spectrum of cotton fabric treated with microcapsules. As oxidation of carbonyl groups creates peaks at around the 1650–1800 cm^{-1} range, the peak at 1734 cm^{-1} may have originated from the vibration of carbonyl groups of gelatin polymer or the C=C stretching of the D-limonene aromatic ring [42, 45]. Both FTIR spectrophotometer and SEM-EDX analyses prove the presence of microcapsules on the cotton fabric, even in small amounts.

Figure 7 shows the FTIR spectra of pure and microcapsule transferred 100% polyester fabrics. As given in reference [49], characteristic peaks of pure polyester fabric are observed at 1408 (aromatic ring), 1340 (carboxylic ester), 1239 (carboxylic ester), 1092 (C-O-C asymmetric stretch), 1017 (O = C-O-C or secondary alcohol groups), 970 (C = C stress), 871 (C-O-C C symmetric stress), 869, 831, 723 and 505 cm^{-1} [50, 51]. There was a strong peak at 1713 cm^{-1} and it depended on the vibration of carbonyl groups. It is known that even slight changes in the intensities of the peaks in the functional group region in the wavelength range of 4000–1500 cm^{-1} indicate the differences created on the material when the amounts and thicknesses of the tested materials are kept constant [52]. In this

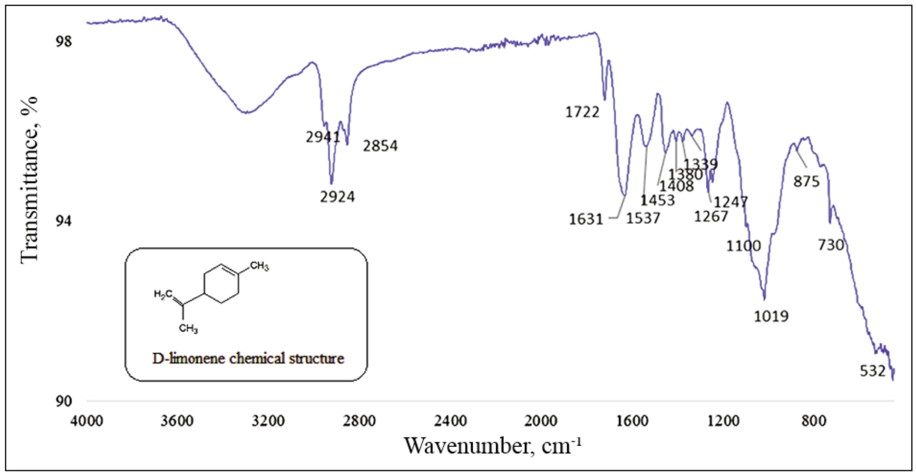

Fig. 5. FTIR spectrum of microcapsules and chemical structure of D-limonene

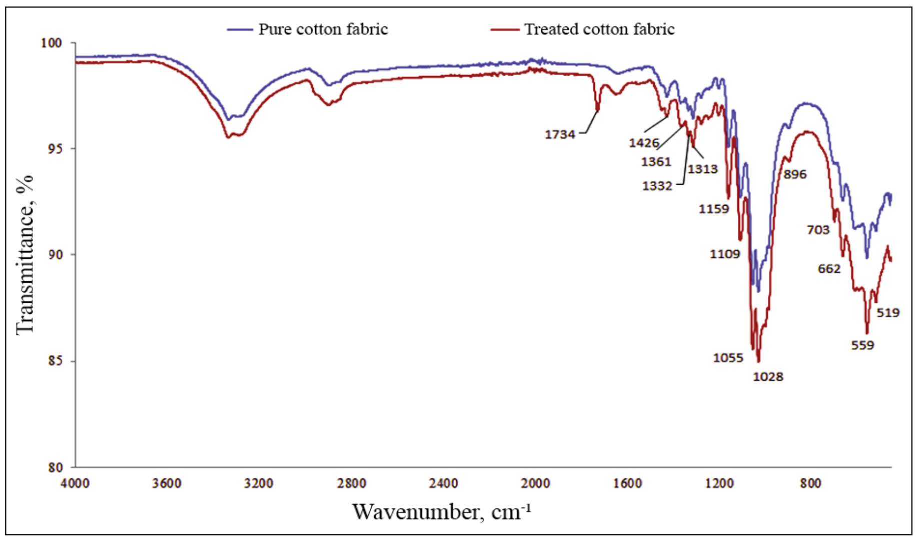

Fig. 6. FTIR spectra of pure and treated cotton fabric

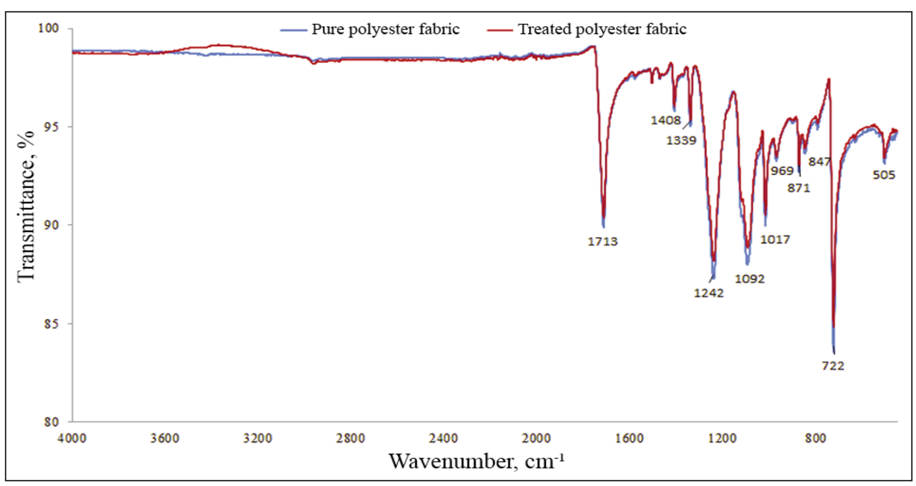

Fig. 7. FTIR spectra of pure and treated polyester fabric

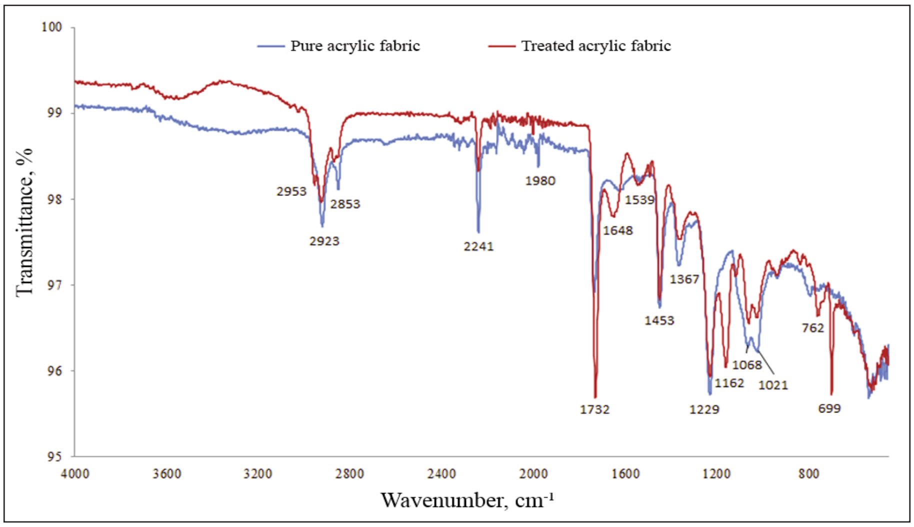

Fig. 8. FTIR spectra of pure and treated acrylic fabric

context, the decrease observed in the strength of the peak at 1713 cm^{-1} in microcapsule transferred fabrics when compared to pure fabric may be elucidated by the interactions that occurred between carbonyl (C = O) groups and microcapsules.

The FTIR spectra of pure and microcapsule transferred fabric 100% acrylic fabrics are given in figure 8. There are two characteristic peaks of acrylic at wavelengths of 1453 cm^{-1} (CH bending) and 1299 cm^{-1} (C-bending). In addition, the strong peak observed at 2241 cm^{-1} is explained by the vibration created by the nitrile groups (-CN) [53]. The presence of microcapsules and the vibration of carbonyl groups in gelatin that form the shell material of microcapsules are responsible for the intensity increase at 1732 cm^{-1}. In addition, the peak at 1539 cm^{-1}, which was not observed in pure fabric, appeared in microcapsule transferred acrylic fabric due to the amide (CONH-) groups. A decrease in the intensity of the peak at 2241 cm^{-1} created due to nitrile groups was observed. The peak at 2953 cm^{-1} created due to the methyl bond vibrations of the microcapsules (namely D-limonene) [45], was observed in the treated acrylic fabric. However, it was not seen in the pure fabric.

Repellency test results

The insect repellent effects of fabrics treated with CPEO microcapsules were evaluated using the excito chamber test unit in this study. Initially, a control test was conducted. When no fabric sample was placed in the exposure chamber and the lid between the chambers was opened, adult *Culex* mosquitoes exhibited flying behaviour only within the exposure chamber and did not move to the escape chamber.

GC-MS analysis identified the three most abundant components of CPEO as D-limonene, p-xylene, and linalool, with retention times of 10.804, 7.44, and 12.194 minutes, respectively. The insect-repellent effects of these components have been reported in the literature [41, 54, 55]. Taking into account the retention times of these components, the waiting period in the test chamber was set to 10–12 minutes. After the waiting period, the number of mosquitoes that moved to the escape chamber was counted. The average mosquito repellency effects for the cotton, polyester, and acrylic fabrics were found to be 80±0%, 80±0%, and 70±0%, respectively. During repellency tests, none of the mosquitoes died but escaped. The repellency effects observed were notably high, aligning with the findings reported in the literature, where repellency efficiencies of plant extracts such as neem, mint, and tulsi leaves ranged from 40% to 90% [36–38].

CONCLUSIONS

In this study, the *C. aurantium* peel essential oil was extracted with a Clevenger apparatus with a 1.7 ml/kg oil yield. The aromatic essential oil was microencapsulated in gelatin and gum arabic polymers via the complex coacervation method at 40 °C initial temperature and with a 1500 rpm stirring speed. The optical microscope images denoted the spherical and uniform microcapsule morphology and reasonable diameter distributions were calculated. The average diameter of the microcapsules was measured as 68.5±34.2 μm. According to the GC-MS analysis, the most abundant aromatic compound of the microcapsules was determined as D-limonene with a percentage of approximately 40% of the total. The SEM-EDX and the FTIR analyses confirmed the successful transfer of microcapsules onto cotton, polyester, and acrylic fabrics. Repellency tests demonstrated that these treated fabrics exhibited 70–80% repellent effects against *Culex* mosquitoes. Future studies may focus on exploring alternative methods for transferring microcapsules onto fabrics, such as impregnation, and developing methods to effectively release the microcapsules through controlled pressure.

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