

# Application of Bioactive Cotton Packages for Packaging and Storage of Grains Using Aromatic Components

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## Abstract

In recent years, Studies into the utility of essential oils and their components in the preservation of food commodities in order to enhance shelf-life has been successfully carried out. These essential oils and their components (Carvacrol, Eugenol and Cinnamaldehyde) can be used as antimicrobials and food preservative agents is of concern because of several reported side effects of synthetic oils. Due to the ability of essential oil constituents to antimicrobial potential it could be used to as a food preservative for grains, cereals, vegetables and fruits. The aim of the treatments were carried out on the treated cotton packaging to maximize the benefit from its antimicrobial activity to extend grain Storage periods. The cotton fabrics treated in three steps: Carboxymethylation of cotton fabric (CMC) Cationization of cotton fabric 3-Treatment of the fabrics by reactive-cyclodextrine (RCD). Modified and unmodified cotton fabrics were treated with antibacterial agent by dipping them at room temperature for 2 hours under stirring in dimethylsulfoxide (DMSO) solution containing 200, 250, 250ppm of euganol, carvacrol and cinnamaldehyde respectively the samples were then roll-squeezed at pick up 100% and dried at room temperature. all treated and untreated fabrics were treated against antimicrobial tests. The types of cotton fabric composite treated by Cinnamaldehyde highest impact on reducing microbial preparation during storage period followed by cotton fabric composite treated by Carvacrol followed by cotton fabric composite treated by Eugenol which is less impactful to reduce microbial count during the storage period.

**Key words:** antimicrobial; bioactive cotton; food package; aromatic components

## Introduction

The antimicrobial packaging consider a tool of active packing. Antimicrobial packaging known as a system which can kill or stop pathogenic and spoilage microorganisms which causes contamination of foods. The new trend in antimicrobial packaging is by adding an antimicrobial agents or by using polymers have antimicrobial properties that satisfy traditional packaging requirements. Antimicrobial packaging substances have to increase the lag phase and lessen the rat of growing of microorganisms to develop the shelf life and maintain the quality and safety of foods 1. The natural antimicrobials show sufficient capacity to reduce microbial contamination inside the food package were reported in some study 2,3. Therefore, the adding of efficient antimicrobials to packaging materials food will improve foodstuff shelf life by removing undesirable pathogens and/or delaying microbial spoilage 4,5. Different antimicrobials have been added to different packaging materials. In particular, the bioactive molecules of essential oils and their components, herbs, and spices have been tested due to their, antiviral antifungal, and

insecticidal properties 4,6. The carvacrol is one of major components of the Labiatae family, including, Satureja, Origanum, Thymus, Thymbra and Coridothymus7,8, has grow for wide-spectrum antimicrobial activity, and it has been scanned by a large number of researchers worldwide. Carvacrol is consider a monoterpenic phenol. Carvacrol is mentioned to have a big set of biological properties, including antifungal, phytotoxic, insecticidal, antioxidant, antitumor, antimutagenic, antiparasitic, and antimicrobial activities7,9,10. Furthermore, carvacrol in the United States and Europe has been confirmed as a safe food additive due to the “generally recognized as safe” status, and it is also used as a flavoring agent in sweets, chewing gum and beverages 2,11. Based on the presiding reasons, the use of carvacrol in active packaging systems is an emerging area with potential to be applied in the food industry 12,13. Cinnamaldehyde is an effective inhibitor of the development of yeasts, bacteria, and molds as well as toxin production by microorganisms. The inhabitation of the accession of a number of bacteria

such as *Bacillus* spp., *Enterobacter sakazakii*, *Enterobacter* spp., *E. coli* O1587:H7, *Escherichia coli*, *Micrococcus* spp., *Listeria innocua*, and *Staphylococcus* spp.14,15,16 and postharvest pathogenic molds including *Penicillium digitatum*. As one of the phenols, eugenol (2-methoxy-4-allylphenol), C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>, is a principal component of clove bud oil (ca. 72-80%). The physical properties of Eugenol is a clove odor, colorless to slightly yellow liquid with a spicy 17. It is also classified as safe material by the FDA. Eugenol appear an inhibitory effect on the growth of *B. cereus*, *Campylobacter jejuni*, *E. coli*, *E. coli* O1587:H7, *P. digitatum*, *L. monocytogenes* *Pseudomonas fluorescens*, *Salmonella enterica*, *S. enteritidis*, and *S. aureus* 18,4. Dorman and Deans 19 reported that eugenol shows the widest spectrum of activity against 24 out of 25 bacteria, except for *Leuconostoc cremoris*. The aim of the present study was to use types of cotton fabric packaging composite treated by eugenol, carvacrol and cinnamaldehyde respectively to maximize the benefit from its antimicrobial activity to extend grain Storage periods.

## 2. Materials and Methods

### Materials

Chemicals: Essential oils (Eo) main compounds (Carvacrol, Eugenol and Cinnamaldehyde) used in this study were obtained from sigma-Aldrich and their purity were more than 98%. Monochlorotriazinyl-β-cyclodextrin, referred here as reactive R-cyclodextrin (RCD), was provided by waker chemic GmbH, Germany. Sodium carbonate, sodium hydroxide, acetic acid, monochloro acetic acid were of laboratory grade chemicals. 3-chloro-2-hydroxypropyl trimethyl ammonium chloride (69%) of technical grade chemical was kindly supplied under the commercial name [Quat188] by Aldrich and Dimethyl sulfoxide [DMSO] were obtained from sigma-Aldrich and their purity were than 99%. Fabrics: Cotton fabric mill de-sized, scoured

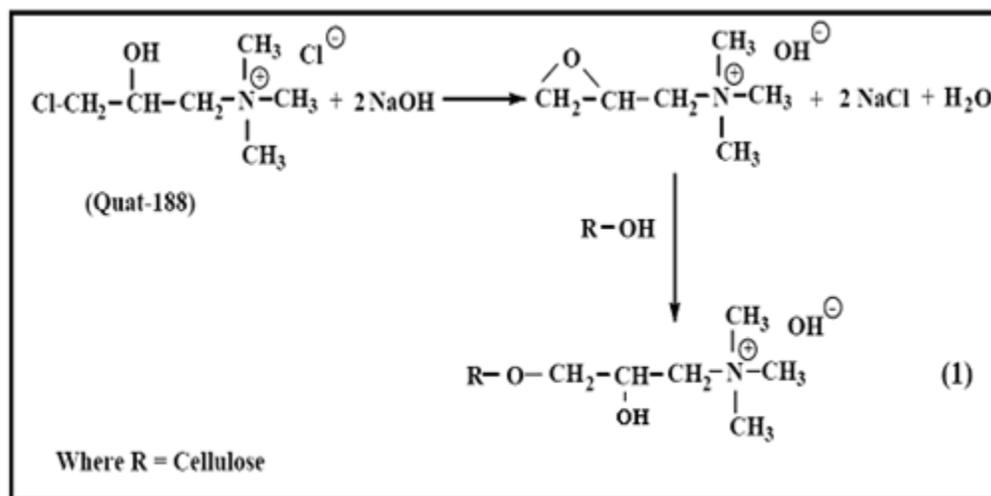
or bleached, plain weave was supplied by Misr Company for spinning and weaving Mehalla El-Kobra, Egypt. The fabric was further purified in the laboratory by washing at 100oC for 60 minutes using a solution containing 2g/L sodium carbonate and nonionic wetting agent (Egyptol). The fabric was then washed several times with boiling water then with running water and finally dried at ambient conditions.

### Methods

In the presented study the cotton fabrics treated in three steps: Carboxymethylation of cotton fabric (CMC) Cationization of cotton fabric 3-Treatment of the fabrics by reactive-cyclodextrine (RCD), Modified and unmodified cotton fabrics were treated with antibacterial agent by dipping them at room temperature for 2 hours under stirring in dimethylsulfoxide (DMSO) solution containing 200, 250, 250ppm of eugenol, carvacrol and cinnamaldehyde respectively the samples were then roll-squeezed at pick up 100% and dried at room temperature. All treated and untreated fabrics were treated against antimicrobial tests.

#### Cationization of cotton fabric

Chemical modification of the cotton fabric through cationization was carried out as per the pad-dry-cure method<sup>20</sup>. The experimental procedures adopted were as follows: 3-Chloro-2-hydroxypropyl trimethyl ammonium chloride (Quat-188) was mixed with sodium hydroxide solution at a NaOH/Quat-188 molar ratio of 2:1. The cotton fabric was padded in this mixture in two dips and two nips, and then squeezed to a wet pick-up of about 100%. The cotton fabric was dried at 40oC for 10min and cured at 120oC for 3min. Thus treated cotton gauze was washed with cold water and 1% acetic acid, followed by several washing cycles and finally dried under the normal laboratory conditions<sup>21</sup>.



**Figure 1:** Chemical modification of the cotton fabric through cationization.

#### Partial Carboxymethylation of cotton gauze (PCMC)

Cotton fabric was partially carboxymethylated to yield (PCMC) by a method similar to those previously reported<sup>22</sup>. PCMC was produced in a two-stage process. The first stage refers to a mercerization process in which, cotton fabric was impregnated with 15 wt. % aqueous NaOH for 5min at room temperature, squeezing to a wet pick up of 100% then dried at 60oC for 5min. Etherification is the second stage in which the alkali treated samples were steeped in aqueous solution of sodium salt of monochloroacetic acid (3mol) for 5min at room temperature. These samples were then squeezed to 100 %

wet pick up, sealed in plastic bags and heated at 80oC for 1h then washed and dried at room temperature.

#### Treatment of the fabrics by reactive-cyclodextrin (RCD) followed by antibacterial agents:

Treatment of cotton fabric, CMC and cationized cotton fabric with reactive-cyclodextrin (RCD) was carried out by pad-dry-cure method according to the following conditions: cotton fabric, CMC and cationized cotton fabric were steeped separately in an aqueous solution containing RCD (100g/l) and 20g/l sodium hydroxide putting in the water bath for 1h at 60oC, at the end

the samples were squeezed to a wet pick up 100% by padding in two dips and two nips. The treated fabrics were then dried at 50°C for 5 min and then cured at 120°C for 3 min. The fabric was washed with cold water containing 1% acetic acid, followed by several washing cycles and finally dried under the normal ambient conditions. Then modified cotton, CMC and cationized fabrics previously prepared were treated with antibacterial agents by dipping them separately under stirring in dimethylsulfoxide (DMSO) solution containing 250ppm of Euganol, Carvacrol and Cinnamaldehyde respectively at room temperature for 2h. The samples were then roll-squeezed at pick up 100% and dried at room temperature 14.

## Testing and analysis

### Mechanical properties test:

The mechanical properties were evaluated by strip method according to ASTM D 5035:2006 using a universal testing machine (INSTRON 4201) at room temperature with crosshead speed of 20 mm/min. The tensile strength and elongation at auto break were measured for both untreated cotton fabrics and those treated fabrics. The samples were cut into strips of 5 cm width and 20 cm length, and every data point is the average of three measurements 23.

### Antibacterial activity test:

The antibacterial properties of the treated cotton fabrics were evaluated according to an American Association of Textile Chemist and Colorists (AATCC) test method 100–2004 24. Two bacteria, Gram-positive bacteria, *Staphylococcus aureus*, abbreviated as *S. aureus*, and Gram-negative bacteria, *Escherichia coli*, abbreviated as *E. coli*, were used. A total of 10µg of the treated cotton fabrics was added to a tube containing 5mL of freshly prepared brain heart infusion broth BHIB (HiMedia, India), which is inoculated with the nominated bacteria (1.6×10<sup>5</sup>/mL). The tubes were incubated at 37°C for 24h in the presence of light source. The turbidity of the test tubes was compared visually to the control BHIB tube. Each tube was diluted, and fractions were plated on Nutrient Agar plates and incubated at 37°C for 24h. Colony forming units/mL was calculated by multiplying the number of colonies by the dilution factor. Antibacterial activities were expressed in terms of the percentage reduction of the microorganisms and calculated by eq 1.

$$\text{Bacterial Reduction (R)\%} = \frac{(A - B)}{A} \times 100 \quad (1)$$

Where A and B are the number of microorganism colonies on untreated and treated cotton fabrics, respectively.

### Microbial count test

Microbiological examination: The microbiological examinations of samples included the determination of total aerobic and anaerobic counts, mould counts, Coliform, and *Bacillus cereus*. Twenty-five grams of each sample were homogenized in 225 mL peptone water (0.1%) using a stomacher model 400 (Seward Laboratory, London, UK) for 1-2 minutes to give a final dilution of 1:10. Samples were then serially diluted and plated using the appropriate medium.

### Total bacteria

Were placed on PCA medium using pour plate technique according to 25 . The inoculated plates were incubated at 35°C for 3 days. The developing colonies were counted, and the Total Aerobic Bacterial counts (TABC) were expressed as colony forming units (CFU) per gram of samples.

### Escherichia coli:

*Escherichia coli* was counted according to the method described by 26 using MCA medium. The plates were incubated at 44°C for 24-48 hr and the suspected colonies were streaked on EMBA and incubated at 44°C for 24 h.

### Total coliform:

plate count agar was poured into the plates 26, evenly distributed and incubated at 37°C for 48 h. Colonies were counted using an illuminated magnifying colony counter. To evaluate any difference between the use of water and saline, this procedure was repeated using sterile saline.

### Bacillus cereus

*Bacillus cereus* was enumerated on MEYPA 27 . The plates were incubated for 16- 24 h at 37°C. Confirmation tests of suspected colonies were biochemically performed by testing acid formation from different sugars.

### Total mold count

Per gram sample were counted on CDYEA medium according to 28 using pour plate technique. Samples were serially diluted, plated and the inoculated plates were incubated at 25°C for 3-5 days and then counted.

## 2.Results And Discussion

Scoured cotton, CMC and cationized cotton fabric were reacted separately with reactive-cyclodextrin (RCD) by pad-dry-cure method by using an aqueous solution containing RCD (100g/l) and 20g/l sodium hydroxide. Then modified cotton, CMC and cationized fabrics were treated separately with antibacterial agents including Euganol, Carvacrol and Cinnamaldehyde as previously described in the experimental part. Mechanical properties of treated and untreated fabrics were evaluated and all the treated and untreated samples were monitored for antibacterial, and Microbial count test. Results obtained along with appropriate discussion is follows.

### Mechanical Properties

Modification of scoured cotton in different conditions for obtaining CMC as well as Cationized fabrics in addition to other treatments with RCD and antimicrobial agents in specific conditions may lead to loss of the tensile strength of the treated fabrics, so mechanical properties of the treated and untreated samples were evaluated due to its importance in storage package properties. Measured properties were the elongation at auto break and tensile strength for the fabrics before and after treatments.

Table 1 shows the mechanical properties of untreated and treated samples with different treatments with RCD or with RCD followed by different antimicrobial agents (Euganol, Carvacrol and Cinnamaldehyde).

Substrate No	Treatment	Mechanical Properties	
		Tensile strength (KgF)	Elongation at auto break (mm)
Blank	Scoured Cotton	81.85 ± 6	11.5 ± 3
1	Cotton treated with RCD	75.65 ± 5	11.0 ± 3
2	Cotton treated with RCD followed by Cinnamaldehyde	74.70 ± 5	10.8 ± 3
3	Cotton treated with RCD followed by Carvacrol	73.95 ± 5	10.8 ± 3

4	Cotton treated with RCD followed by Eugenol	74.15 ± 5	10.9 ± 3
5	CMC	75.50 ± 4	10.6 ± 3
6	CMC treated with CD	73.75 ± 4	10.5 ± 3
7	CMC treated with CD followed by Cinnamaldehyde	73.90 ± 4	10.6 ± 3
8	CMC treated with CD followed by Carvacrol	72.65 ± 4	10.2 ± 2
9	CMC treated with CD followed by Eugenol	74.10 ± 4	10.5 ± 2
10	Cationized Cotton	72.80 ± 4	10.25 ± 2
11	Cationized Cotton treated with CD	72.15 ± 4	10.25 ± 2
12	Cationized Cotton treated with CD followed by Cinnamaldehyde	71.85 ± 4	10.00 ± 2
13	Cationized Cotton treated with CD followed by Carvacrol	71.25 ± 4	9.80 ± 2
14	Cationized Cotton treated with CD followed by Eugenol	71.0 ± 4	9.80 ± 2

**Table I:** Effect of treatment type on the Mechanical properties of cotton fabrics.

All The values are means of triplicate ±SD

It is observed that the untreated sample has elongation around 11.5mm and tensile strength of 81.85kgF. After treatment with RCD or with RCD followed by different antimicrobial agents (Euganol, Carvacrol and Cinnamaldehyde), followed by drying and curing, the elongation and tensile strength marginally decrease and reach 10.8mm and 73.95kgF, respectively indicating that the treatment causes a loss in the elongation at break by 6% and tensile strength by about 9%, for CMC treated fabrics the elongation and tensile strength marginally decrease and reach 10.2mm and 72.65kgF, respectively indicating that the treatment causes a loss in the elongation at break by 11% and tensile strength by about 11%, for cationized treated fabrics the elongation and tensile strength marginally decrease and reach 9.80mm and 71.0kgF, respectively indicating that the treatment causes a loss

in the elongation at break by 15% and tensile strength by about 14% and these losses percentage are practically acceptable for all treated samples and proved that such treatments does not significantly damage the strength properties of cotton 5,14,20.

#### Antibacterial properties

Antibacterial activities of the treated and untreated cotton fabric were determined against two kind of bacteria, namely Staphylococcus aureus (S. aureus) (as gram-positive bacteria) and Escherichia coli (E. coli) (as gram-negative bacteria) according to Agar Diffusion Method (AATCC Test Method 100-2004) 24. Inhibition zone diameter formed around the test samples were taken as a measure for antimicrobial activity. Results obtained are set out in Table 2.

Substrate No	Treatment	Inhibition zone (mm)	
		<i>E. coli</i> ( <i>G</i> <sup>-ve</sup> )	<i>S. aureus</i> ( <i>G</i> <sup>+ve</sup> )
Blank	Scoured Cotton	0.0	0.0
1	Cotton treated with RCD	19	21
2	Cotton treated with RCD followed by Cinnamaldehyde	26	28
3	Cotton treated with RCD followed by Carvacrol	25	26
4	Cotton treated with RCD followed by Eugenol	24	26
5	CMC	15	17
6	CMC treated with CD	19	21
7	CMC treated with CD followed by Cinnamaldehyde	24	27
8	CMC treated with CD followed by Carvacrol	24	25
9	CMC treated with CD followed by Eugenol	22	24
10	Cationized Cotton	19	21
11	Cationized Cotton treated with CD	24	26
12	Cationized Cotton treated with CD followed by Cinnamaldehyde	29	30
13	Cationized Cotton treated with CD followed by Carvacrol	26	28
14	Cationized Cotton treated with CD followed by Eugenol	26	27

**Table 2:** Effect of treatment type on the antibacterial properties of cotton fabrics.

- (i) Untreated scoured fabrics did not show any antimicrobial properties towards *S. aureus* or *E. Coli*.
- (ii) All cotton substrates CMC or cationized cotton shows antimicrobial properties towards *S. aureus* or *E. Coli*.
- (iii) Different treatments of cotton fabrics with RCD or with RCD and oils extract enhances its antibacterial activity towards both *S. aureus* and *E. Coli* where the inhibition zone RCD treated cotton fabric shows 19 and 21mm for *E. Coli* and *S. aureus* respectively, presence of oils with RCD lead to increase the

inhibition zone which increases to 26 and 28mm for Cinnamaldehyde oil and for Carvacrol oil increases to 25 and 26mm while increases with Eugenol oil to 24 and 26 for. E. Coli and S. aureus respectively proving the improvements of antibacterial properties for the treated cotton fabrics.

- (iv) Carboxymethylated cotton (CMC) show inhibition zone 15, 17 towards E. Coli and S. aureus respectively, the treatments of (CMC) with RCD or with RCD and oils extract enhances its antibacterial activity towards both S. aureus and E. Coli where the inhibition zone RCD treated CMC fabric shows 19 and 21mm for E. Coli and S. aureus respectively, presence of oils with RCD lead to increase the inhibition zone which increases to 24 and 27mm for Cinnamaldehyde oil and for Carvacrol oil increases to 24 and 25mm while increases with Eugenol oil to 22 and 24 for. E. Coli and r S. aureus respectively proving the improvements of antibacterial properties for the treated CMC fabrics.
- (v) Cationized cotton show inhibition zone 19, 21 towards E. Coli and S. aureus respectively, the treatments of cationized cotton with RCD or with RCD and oils extract enhances its antibacterial activity towards both S. aureus and E. Coli where the inhibition zone RCD treated cationized fabric shows 24 and 26mm for E. Coli and S. aureus respectively, presence of oils with RCD lead to increase the inhibition zone which increases to 29 and 30mm for Cinnamaldehyde oil and for Carvacrol oil increases to 26 and 28mm while increases with Eugenol oil to 26 and 27 for. E. Coli and r S. aureus respectively proving the improvements of antibacterial properties for the treated cationized fabrics.
- (vi) All the treated fabrics indicates enhancement in its antibacterial activity towards both S. aureus and E. Coli and the inhibition zone of treated cationized cotton with RCD followed by cinnamaldehyde is the best between all the treated fabrics. These results obtained similar than those obtained by Hashem et al.,29.

A known weight from Egyptian wheat were stored in a package made from treated cotton package for 9 months and the microbial count was monitored 0,1,3, 6 and 9 month. In this report, we will present the data obtained during 9 months. The package content was monitored for Total anaerobic bacteria count, Total mould count, Coliform and Bacillus cereus. Microbial count results for wheat storage period from one month to nine months in various treatments of cotton fabric which has been treated in the three volatile active compounds (Carvacrol, Eugenol and Cinnamaldehyde) showed that the cotton fabric treatment which named (cationized+cyclodextrin with three volatile active compounds) is the highest impact in reducing microbial count during storage period followed by cotton fabric (CMC+cyclodextrin with three volatile active compounds) followed by (Cotton+cyclodextrin with three volatile active compounds) which is less impactful to reduce microbial count. The types of cotton fabric composite treated by Cinnamaldehyde highest impact on reducing microbial preparation during storage period followed by cotton fabric composite treated by Carvacrol followed by cotton fabric composite treated by Eugenol which is less impactful to reduce microbial count during the storage period tables (3, 4, and 5). Also, all treatments were the results of counting at zero time. These results can be supported by the finding of Sanla-Ead et al. 30 who reported that the cinnamaldehyde and eugenol had 'moderate-strong inhibitory' and 'strong-highly solid inhibitory' qualities, individually. Additionally, shows the potential utilization of cinnamaldehyde and eugenol for application in antimicrobial bundling film or covering. In this examination cinnamaldehyde and eugenol were explored for their antimicrobial movement against 10 pathogenic and decay microbes and three strains of yeast. Cinnamaldehyde-consolidated and eugenol-joined methyl cellulose films were set up to acquire dynamic antimicrobial bundling materials. Also, (Bnyan et al. 24 showed that the inhibition effect of carvacrol in various grouping of bacterial development were an extraordinary hindrance development on completely considered bacterial segregates aside from Pseudomonas aeruginosa.

**Results of microbial count test**

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
<b>All Treatments</b>	<b>0</b>	<b>9 × 10<sup>4</sup></b>	<b>7 × 10<sup>8</sup></b>	<b>8 × 10<sup>4</sup></b>	<b>6 × 10<sup>5</sup></b>	<b>9 × 10<sup>6</sup></b>
<b>Cotton treated with CD</b>	<b>1</b>	<b>9 × 10<sup>4</sup></b>	<b>7 × 10<sup>8</sup></b>	<b>8 × 10<sup>4</sup></b>	<b>6 × 10<sup>5</sup></b>	<b>9 × 10<sup>6</sup></b>
	<b>3</b>	<b>9 × 10<sup>4</sup></b>	<b>8 × 10<sup>8</sup></b>	<b>8 × 10<sup>4</sup></b>	<b>6 × 10<sup>5</sup></b>	<b>9 × 10<sup>6</sup></b>
	<b>6</b>	<b>8 × 10<sup>4</sup></b>	<b>9 × 10<sup>8</sup></b>	<b>7 × 10<sup>4</sup></b>	<b>5 × 10<sup>5</sup></b>	<b>8 × 10<sup>6</sup></b>
	<b>9</b>	<b>8 × 10<sup>4</sup></b>	<b>1 × 10<sup>9</sup></b>	<b>7 × 10<sup>4</sup></b>	<b>5 × 10<sup>5</sup></b>	<b>8 × 10<sup>6</sup></b>
<b>Cotton treated with CD followed by Cinnamaldehyde</b>	<b>1</b>	<b>6 × 10<sup>4</sup></b>	<b>5 × 10<sup>8</sup></b>	<b>5 × 10<sup>4</sup></b>	<b>4 × 10<sup>5</sup></b>	<b>6 × 10<sup>6</sup></b>
	<b>3</b>	<b>5 × 10<sup>4</sup></b>	<b>4 × 10<sup>8</sup></b>	<b>3 × 10<sup>4</sup></b>	<b>3 × 10<sup>5</sup></b>	<b>5 × 10<sup>6</sup></b>
	<b>6</b>	<b>2 × 10<sup>4</sup></b>	<b>2 × 10<sup>8</sup></b>	<b>2 × 10<sup>4</sup></b>	<b>2 × 10<sup>5</sup></b>	<b>3 × 10<sup>6</sup></b>
	<b>9</b>	<b>1 × 10<sup>4</sup></b>	<b>1 × 10<sup>8</sup></b>	<b>1 × 10<sup>4</sup></b>	<b>1 × 10<sup>5</sup></b>	<b>2 × 10<sup>6</sup></b>
<b>Cotton treated with CD followed by Carvacrol</b>	<b>1</b>	<b>7 × 10<sup>4</sup></b>	<b>6 × 10<sup>8</sup></b>	<b>6 × 10<sup>4</sup></b>	<b>5 × 10<sup>5</sup></b>	<b>7 × 10<sup>6</sup></b>
	<b>3</b>	<b>6 × 10<sup>4</sup></b>	<b>5 × 10<sup>8</sup></b>	<b>4 × 10<sup>4</sup></b>	<b>4 × 10<sup>5</sup></b>	<b>6 × 10<sup>6</sup></b>
	<b>6</b>	<b>4 × 10<sup>4</sup></b>	<b>3 × 10<sup>8</sup></b>	<b>3 × 10<sup>4</sup></b>	<b>3 × 10<sup>5</sup></b>	<b>5 × 10<sup>6</sup></b>
	<b>9</b>	<b>3 × 10<sup>4</sup></b>	<b>2 × 10<sup>8</sup></b>	<b>2 × 10<sup>4</sup></b>	<b>2 × 10<sup>5</sup></b>	<b>4 × 10<sup>6</sup></b>
<b>Cotton treated with CD followed by Eugenol</b>	<b>1</b>	<b>8 × 10<sup>4</sup></b>	<b>7 × 10<sup>8</sup></b>	<b>7 × 10<sup>4</sup></b>	<b>6 × 10<sup>5</sup></b>	<b>8 × 10<sup>6</sup></b>
	<b>3</b>	<b>7 × 10<sup>4</sup></b>	<b>6 × 10<sup>8</sup></b>	<b>5 × 10<sup>4</sup></b>	<b>5 × 10<sup>5</sup></b>	<b>7 × 10<sup>6</sup></b>
	<b>6</b>	<b>5 × 10<sup>4</sup></b>	<b>4 × 10<sup>8</sup></b>	<b>4 × 10<sup>4</sup></b>	<b>4 × 10<sup>5</sup></b>	<b>6 × 10<sup>6</sup></b>
	<b>9</b>	<b>3 × 10<sup>4</sup></b>	<b>3 × 10<sup>8</sup></b>	<b>3 × 10<sup>4</sup></b>	<b>3 × 10<sup>5</sup></b>	<b>5 × 10<sup>6</sup></b>

**Table 3:** Results of antimicrobial count test of cotton substrates in which Egyptian Wheat stored for different periods.

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
All Treatments	0	$9 \times 10^4$	$7 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
Carboxymethylated Cotton (CMC) treated with CD	1	$9 \times 10^4$	$8 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	3	$9 \times 10^4$	$9 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	6	$8 \times 10^4$	$9 \times 10^8$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
	9	$8 \times 10^4$	$1 \times 10^9$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
Carboxymethylated Cotton (CMC) treated with CD followed by Cinnamaldhyde	1	$5 \times 10^4$	$4 \times 10^8$	$4 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	3	$4 \times 10^4$	$3 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$4 \times 10^6$
	6	$1 \times 10^4$	$1 \times 10^8$	$1 \times 10^4$	$1 \times 10^5$	$2 \times 10^6$
	9	$9 \times 10^3$	$8 \times 10^7$	$9 \times 10^3$	$8 \times 10^4$	$1 \times 10^6$
Carboxymethylated Cotton (CMC) treated with CD followed by Carvacrol	1	$6 \times 10^4$	$5 \times 10^8$	$5 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	3	$5 \times 10^4$	$4 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	6	$3 \times 10^4$	$2 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$4 \times 10^6$
	9	$2 \times 10^4$	$1 \times 10^8$	$1 \times 10^4$	$1 \times 10^5$	$3 \times 10^6$
Carboxymethylated Cotton (CMC) treated with CD followed by Eugenol	1	$7 \times 10^4$	$6 \times 10^8$	$6 \times 10^4$	$5 \times 10^5$	$7 \times 10^6$
	3	$6 \times 10^4$	$5 \times 10^8$	$5 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	6	$4 \times 10^4$	$3 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	9	$2 \times 10^4$	$2 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$4 \times 10^6$

**Table 4:** Results of antimicrobial count test of Carboxymethylated cotton substrates in which Egyptian Wheat stored for different periods.

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
All Treatments	0	$9 \times 10^4$	$7 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
Cationized Cotton treated with CD	1	$9 \times 10^4$	$8 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	3	$9 \times 10^4$	$9 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	6	$8 \times 10^4$	$9 \times 10^8$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
	9	$8 \times 10^4$	$1 \times 10^9$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
Cationized Cotton treated with CD followed by Cinnamaldhyde	1	$1 \times 10^4$	$1 \times 10^8$	$2 \times 10^4$	$1 \times 10^5$	$3 \times 10^6$
	3	$7 \times 10^3$	$6 \times 10^7$	$8 \times 10^3$	$8 \times 10^4$	$1 \times 10^6$
	6	$6 \times 10^3$	$3 \times 10^7$	$5 \times 10^3$	$7 \times 10^4$	$6 \times 10^5$
	9	$3 \times 10^3$	$6 \times 10^6$	$1 \times 10^3$	$3 \times 10^4$	$2 \times 10^5$
Cationized Cotton treated with CD followed by Carvacrol	1	$3 \times 10^4$	$3 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$4 \times 10^6$
	3	$2 \times 10^4$	$2 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$3 \times 10^6$
	6	$1 \times 10^4$	$1 \times 10^8$	$8 \times 10^3$	$9 \times 10^4$	$1 \times 10^6$
	9	$8 \times 10^3$	$8 \times 10^7$	$5 \times 10^3$	$7 \times 10^4$	$8 \times 10^5$
Cationized Cotton treated with CD followed by Eugenol	1	$4 \times 10^4$	$4 \times 10^8$	$4 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	3	$3 \times 10^4$	$3 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	6	$2 \times 10^4$	$2 \times 10^8$	$1 \times 10^4$	$2 \times 10^5$	$3 \times 10^6$
	9	$1 \times 10^4$	$1 \times 10^8$	$9 \times 10^3$	$8 \times 10^4$	$1 \times 10^6$

**Table 5:** Results of antimicrobial count test of Cationized cotton substrates in which Egyptian Wheat stored for different periods.

Various investigators have used essential oils and their components, either in pure or formulation forms, to enhance the shelf-life of food commodities in different storage containers such as those made of cardboard, tin, glass, polyethylene, or natural fabrics and have observed significant enhancement of shelf-life 20. An earlier study reported that some essential oil constituents such as carvacrol, eugenol, cinnamaldehyde, citronellol, farnesol, and nerol capable of protecting chili fruits and seeds from fungal infection for up to 6 months 21,31. The use of Cymbopogon pendulous essential oil as a fumigant increased groundnut shelf-life by 6–12 months 32, which proves to be more

effective than *P. roxburghii* essential oil. These differences in efficacy of essential oils components may be related to the use of oils from different plant species, as well as to their chemical composition, dose level, and storage container type. Bioactivity may be associated with the presence of high content of cinnamaldehyde in the composition of essential oils. Therefore, these major components may be considered as possible sources for the development of new antimicrobial agents and may be used in synergy with currently available synthetic antibiotics or antimicrobials. In addition, the cinnamaldehyde molecule is promising as a prototype for derivatives

with antibacterial properties and extended antibiotics 33. The all results were arranged from highest to lowest impact effect on the microbial count in table presented for illustrate and comparison between different treatments and (6).

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
<b>All Treatments</b>	<b>0</b>	$9 \times 10^4$	$7 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
<b>Cotton treated with RCD</b>	<b>1</b>	$9 \times 10^4$	$7 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	<b>3</b>	$9 \times 10^4$	$8 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	<b>6</b>	$8 \times 10^4$	$9 \times 10^8$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
	<b>9</b>	$8 \times 10^4$	$1 \times 10^9$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
<b>CMC treated with RCD</b>	<b>1</b>	$9 \times 10^4$	$8 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	<b>3</b>	$9 \times 10^4$	$9 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	<b>6</b>	$8 \times 10^4$	$9 \times 10^8$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
	<b>9</b>	$8 \times 10^4$	$1 \times 10^9$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
<b>Cationic treated with RCD</b>	<b>1</b>	$9 \times 10^4$	$8 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	<b>3</b>	$9 \times 10^4$	$9 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
	<b>6</b>	$8 \times 10^4$	$9 \times 10^8$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
	<b>9</b>	$8 \times 10^4$	$1 \times 10^9$	$7 \times 10^4$	$5 \times 10^5$	$8 \times 10^6$
<b>Cotton treated with RCD followed by Carvacrol</b>	<b>1</b>	$7 \times 10^4$	$6 \times 10^8$	$6 \times 10^4$	$5 \times 10^5$	$7 \times 10^6$
	<b>3</b>	$6 \times 10^4$	$5 \times 10^8$	$4 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	<b>6</b>	$4 \times 10^4$	$3 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	<b>9</b>	$3 \times 10^4$	$2 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$4 \times 10^6$
<b>CMC treated with RCD followed by Carvacrol</b>	<b>1</b>	$6 \times 10^4$	$5 \times 10^8$	$5 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	<b>3</b>	$5 \times 10^4$	$4 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	<b>6</b>	$3 \times 10^4$	$2 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$4 \times 10^6$
	<b>9</b>	$2 \times 10^4$	$1 \times 10^8$	$1 \times 10^4$	$1 \times 10^5$	$3 \times 10^6$
<b>Cationized Cotton treated with RCD followed by Carvacrol</b>	<b>1</b>	$3 \times 10^4$	$3 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$4 \times 10^6$
	<b>3</b>	$2 \times 10^4$	$2 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$3 \times 10^6$
	<b>6</b>	$1 \times 10^4$	$1 \times 10^8$	$8 \times 10^3$	$9 \times 10^4$	$1 \times 10^6$
	<b>9</b>	$8 \times 10^3$	$8 \times 10^7$	$5 \times 10^3$	$7 \times 10^4$	$8 \times 10^5$

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
<b>All Treatments</b>	<b>0</b>	$9 \times 10^4$	$7 \times 10^8$	$8 \times 10^4$	$6 \times 10^5$	$9 \times 10^6$
<b>Cotton treated with RCD followed by Eugenol</b>	<b>1</b>	$8 \times 10^4$	$7 \times 10^8$	$7 \times 10^4$	$6 \times 10^5$	$8 \times 10^6$
	<b>3</b>	$7 \times 10^4$	$6 \times 10^8$	$5 \times 10^4$	$5 \times 10^5$	$7 \times 10^6$
	<b>6</b>	$5 \times 10^4$	$4 \times 10^8$	$4 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	<b>9</b>	$3 \times 10^4$	$3 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
<b>CMC treated with RCD followed by Eugenol</b>	<b>1</b>	$7 \times 10^4$	$6 \times 10^8$	$6 \times 10^4$	$5 \times 10^5$	$7 \times 10^6$
	<b>3</b>	$6 \times 10^4$	$5 \times 10^8$	$5 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	<b>6</b>	$4 \times 10^4$	$3 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	<b>9</b>	$2 \times 10^4$	$2 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$4 \times 10^6$
<b>Cationic fabric treated with RCD followed by Eugenol</b>	<b>1</b>	$4 \times 10^4$	$4 \times 10^8$	$4 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	<b>3</b>	$3 \times 10^4$	$3 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	<b>6</b>	$2 \times 10^4$	$2 \times 10^8$	$1 \times 10^4$	$2 \times 10^5$	$3 \times 10^6$
	<b>9</b>	$1 \times 10^4$	$1 \times 10^8$	$9 \times 10^3$	$8 \times 10^4$	$1 \times 10^6$
	<b>1</b>	$6 \times 10^4$	$5 \times 10^8$	$5 \times 10^4$	$4 \times 10^5$	$6 \times 10^6$
	<b>3</b>	$5 \times 10^4$	$4 \times 10^8$	$3 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$

Cotton treated with CD followed by Cinnamaldhyde	6	$2 \times 10^4$	$2 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$3 \times 10^6$
	9	$1 \times 10^4$	$1 \times 10^8$	$1 \times 10^4$	$1 \times 10^5$	$2 \times 10^6$
CMC treated with RCD followed by Cinnamaldhyde oil	1	$5 \times 10^4$	$4 \times 10^8$	$4 \times 10^4$	$3 \times 10^5$	$5 \times 10^6$
	3	$4 \times 10^4$	$3 \times 10^8$	$2 \times 10^4$	$2 \times 10^5$	$4 \times 10^6$
	6	$1 \times 10^4$	$1 \times 10^8$	$1 \times 10^4$	$1 \times 10^5$	$2 \times 10^6$
	9	$9 \times 10^3$	$8 \times 10^7$	$9 \times 10^3$	$8 \times 10^4$	$1 \times 10^6$
Cationized Cotton treated with RCD followed by Cinnamaldhyde	1	$1 \times 10^4$	$1 \times 10^8$	$2 \times 10^4$	$1 \times 10^5$	$3 \times 10^6$
	3	$7 \times 10^3$	$6 \times 10^7$	$8 \times 10^3$	$8 \times 10^4$	$1 \times 10^6$
	6	$6 \times 10^3$	$3 \times 10^7$	$5 \times 10^3$	$7 \times 10^4$	$6 \times 10^5$
	9	$3 \times 10^3$	$6 \times 10^6$	$1 \times 10^3$	$3 \times 10^4$	$2 \times 10^5$

**Table 6:** Comparison between all substrates in the presence of RCD and RCD+Carvacrol, Eugenol and Cinnamaldhyde.

## Conclusion

Carvacrol, Eugenol and Cinnamaldehyde as essential oil constituents have pronounced antimicrobial and food preservative properties that have great significance in the food industry. Along these lines, the different properties of essential oil constituents offer the chance of utilizing normal, safe, eco-accommodating, practical, inexhaustible, and effectively biodegradable antimicrobials for food product protection sooner rather than later. The types of cotton fabric composite treated by Cinnamaldehyde highest impact on reducing microbial preparation during storage period followed by cotton fabric composite treated by Carvacrol followed by cotton fabric composite treated by Eugenol which is less impactful to reduce microbial count during the storage period.

**Competing Interests:** The authors declare that they have no competing interests.

**Authors' Contributions:** All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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