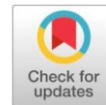


## Research Article

## Open Access

**Antimicrobial properties of green zinc oxide nano-coated cotton fabrics****Putta Aparna and Jyoti. V. Vastrad**

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**ABSTRACT**

The fruit processing sector disposes of large amounts of fruit waste in landfills or rivers, posing environmental risks. As a result, recycling and producing livestock feed resources, as well as extracting or developing value-added products, are required. Consequently, we utilize the biomass of *C. reticulata* fruit peel in the environmentally friendly production of zinc oxide nanoparticles. In addition, a nanoparticle coating on fabrics will have a highly active surface with different functional properties. In the parallel streak method, cotton fabrics treated with zinc oxide nanoparticles using citrus *reticulata* peel powder had the best antibacterial efficacy against *E. coli* and *S. aureus*. The results show that fabrics treated with ZnO-1 have stronger antibacterial activity than fabrics treated with ZnO-2, while untreated fabrics have no antibacterial activity. The wash durability test results showed a highly significant difference at a 1 percent significant level in numerous washings, and the proportion of bacterial reduction dropped as the wash cycle lengthened.

**Keywords:** ZnO, zinc oxide, *E.Coli*, *S.Aureus*, nano-coated, cotton fabric, cross-linking agent, Zone of inhibition

**Introduction**

Nanotechnology is a rapidly expanding interdisciplinary field that is frequently considered a new industrial revolution. It can be defined as research for the design, synthesis, and manipulation of the structure of particles with dimensions smaller than 100 nm. Nanotechnology is based on the fact that the properties of materials change significantly as their dimensions are reduced to the nanoscale. Recently, the textile industry has recognized the significance of nanotechnology. Therefore, nanotechnology in textiles can be defined as the understanding, manipulating, and controlling of matter at the above-mentioned length, to exploit the physical, chemical, and biological properties to develop the next generation of enhanced materials, devices, structures, and systems [22].

Orange peel, which is the primary waste fraction in the production of the food industry, contains fiber, vitamin C, folate, vitamin B6, calcium and other essential nutrients. Orange skin contains polyphenols, which protect against a variety of diseases (30). Orange peel is beneficial for healthy skin as it possesses anti-microbial, anti-inflammatory, anti-fungal and anti-oxidant properties with active phytochemicals such as flavonoids, vitamins, terpenoids, carotenoids, saponins, lignin, and plant sterols [13].

Large amounts of fruit waste from the fruit processing sector are typically disposed of in landfills or rivers, posing environmental risks. As a result, recycling and producing livestock feed resources, as well as extracting or developing value-added products, are required [25]. Thus, fruit waste generation not only results in financial losses but also adds to the expense of waste management and disposal.

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Indeed, it is often assumed that the adoption of efficient technology and careful handling during various procedures can reduce losses by more than 50 per cent [10]. As a result, the biomass of *C. reticulata* fruit peel is utilized in the green production of zinc oxide nanoparticles.

Zinc oxide nanoparticles have an extremely large relative surface area, thus increasing their contact with bacteria or fungi and vastly improving their bactericidal and fungicidal effectiveness. Nano-ZnO is very reactive with proteins, when it contacts bacteria and fungus, it will adversely affect cellular metabolism and inhibit cell growth. It also suppresses respiration, basal metabolism of the electron transfer system and the transport of the substrate into the microbial cell membrane. Different researchers have noticed that phytochemicals present in the orange peel can act as larvicidal, insect development regulators, repellents, and ovipositional attractants, with deterrent properties [7].

Nanotechnology partakes in the real commercial potential of the textile industry. This is mainly because conventional methods used to impart different properties to fabrics often do not lead to permanent effects and they will lose their functions after laundering or wearing. Nanotechnology can provide high durability for fabrics because nanoparticles have a large surface area-to-volume ratio and high surface energy, thus providing a better affinity for fabrics and increasing the durability of the function. In addition, a coating of nanoparticles on fabrics will not affect their breathability or hand feel. Textiles that have been developed with nanotechnology are already used in sports, cosmetics, space technology, clothing and material technologies to protect people better in extreme environments [29]. Nanostructures also have the potential to improve the physical, mechanical, and functional qualities of conventional fabrics. The new concepts exploited for the development of nano finishes have opened up exciting opportunities to hold an enormously promising future for textiles. Therefore, coating the surface of textiles and clothing with green nanoparticles is one approach to produce a highly active surface that has different functional

properties such as UV-blocking, antimicrobial, self-cleaning properties and other properties.

Keywords: Green synthesis, Zinc oxide, Nanoparticles, Antimicrobial, *E.Coli*, *S.Aureus*, Washdurability, *Citrus reticulata peel*

## 2. Materials & Methods

### 2.1 Selection of fabric

Cotton fabric is a type of natural cloth created out of the cotton plant's fibers. It comes in a wide range of weaves and is the most versatile and popular fabric in the world. India is the largest producer of cotton in the world, accounting for about 22 per cent of the world's cotton production. Cotton is a breathable fabric that absorbs heat and perspiration from our bodies and allows them to pass through the fabric to the air outside making it comfortable to the wearer. Thus, the medium-weight, pure cotton plain-weaved fabric was used for the application of green synthesized zinc nanoparticles using *citrus reticulata peel* extract with zinc acetate and zinc nitrate as precursors.

### 2.2 Green synthesis of zinc oxide nanoparticles

For the green synthesis of ZnO nanoparticles, 0.04 M of zinc acetate dihydrate was dissolved in 80 ml of milli-Q water and stirred for four hours by adding *C. reticulata peel* aqueous extract. Sodium hydroxide was added drop by drop to maintain the pH [5].

Similarly, 0.03M of zinc nitrate hexahydrate precursor was dissolved in 80 ml milli-Q water and stirred for five hours by adding *C. reticulata peel* aqueous extract for the synthesis of zinc oxide nanoparticles. To maintain the pH of the solution sodium hydroxide was added dropwise [12].

### 2.3 Desizing and scouring

The following recipe was used for one bath scouring and desizing the cotton fabrics. At elevated temperatures, a hydroxide compound and caustic soda mixture can effectively combine desizing and scouring conditions. Desizing is the process of removing the sizing materials present in cotton fabric to make it suitable for further processing.

#### Recipe

Sodium hydroxide - 2 % owf

Sodium carbonate - 2 % owf

Turkey red oil - 2 % owf

Material to Liquor Ratio (MLR) - 1: 20

Time - 85 min

Temperature - 80 to 90 °C

The fabric was weighed and the quantities of sodium hydroxide, sodium carbonate, turkey red oil and Material Liquor Ratio (MLR) were calculated. Water was mixed with sodium hydroxide and sodium carbonate and the solution was heated to 80°. The fabric was then immersed in the solution and treated for 85 minutes at temperatures ranging from 80 to 90 °. After that, the fabric was thoroughly washed under running water to remove any traces of sodium hydroxide and shade-dried.

### 2.4 Pad-dry-cure method

The pad-dry-cure method is a widely used technique for textile finishing and can be used to add a variety of coatings. The fabric was submerged in the nano-coating solution and then passed between the rollers in the padding mangle to squeeze out the excess liquid. The finished fabric is then dried and cured. The procedure followed is mentioned below:

#### Recipe

Material to Liquor Ratio (MLR) - 1:20

Synthesized Zinc oxide NPs - 4 % stock solution

Citric acid/Maleic acid - 2, 4, 6 % each owf

The fabric was immersed in the ZnO nano solution containing both crosslinking agents separately for ten minutes and passed through pneumatic padding mangle (R. B. Electronic and Engineering, Mumbai) at a speed of 3 m/min with a pressure of 1kg/ cm<sup>2</sup> to remove excess solution ensuring the wet pick up of 70 per cent [17]. Later, the fabric was shade dried and cured for 10 minutes at 40°C, 50°C and 60°C.

### 2.5 Application of nanoparticles on the cotton fabric

The selected cotton fabric was desized and scoured. Using the pad dry curing method, samples of fabric measuring 25 x 25 cm were prepared and treated with a 4 percent stock of nano-solutions for both the precursors by following the following parameters.

#### Parameters and variables for coating cotton fabrics with green ZnO Nps

Parameters	Variables
Nano concentrations (4 %)	Zinc acetate dihydrate and zinc nitrate hexahydrate
Method of application	Pad-Dry-Cure
Eco-friendly Cross- linking agents	Citric acid and Maleic acid
Cross- linking agents (%)	2,4,6
Curing temperature	40 °C, 50 °C, 60°C

### 2.6 Antimicrobial properties

With the growth of the world population and the spread of disease, the number of antibiotic-resistant microorganisms is rising along with the occurrence of infections from these microorganisms. With this increase in health awareness, many people have focused their attention on educating and protecting themselves against harmful pathogens. It soon became more important for antimicrobial finished textiles to protect the wearer from bacteria than it was to simply protect the garment from fiber degradation. The need for antimicrobial textiles goes hand-in-hand with the rise in resistant strains of microorganisms. Functional textiles include everything from antimicrobial finished textiles to durable or permanent press finished garments, to textiles with self-cleaning properties, and also textiles with nanotechnology. With the above background information, green synthesized ZnO nanoparticles were coated onto cotton fabrics to assess antimicrobial function and finally evaluate the finished fabrics in terms of antibacterial and wash durability [18].

#### 2.6.1 Selection of Bacteria

*Staphylococcus aureus* and *Escherichia coli* are the most common cloth-damaging bacteria. *S. aureus* contaminates undergarments and can cause infections like furuncles and boils, diseases of the skin, etc. *E. coli* is non-pathogenic in normal conditions, but if it is present in excess, it will become the causative agent of various diseases like urinary tract infection, diarrhea, and vomiting. Because of this, the study used gram-positive *Staphylococcus aureus* (ATCC 6538) and gram-negative *Escherichia coli* (ATCC 8739).

##### 2.6.1.1 *Staphylococcus aureus*

*Staphylococcus aureus* is a gram-positive round-shaped bacterium, a member of the firmicutes and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow

without the need for oxygen. Although *S. aureus* usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of cell-surface proteins that bind and inactivate antibodies. *S. aureus* is one of the leading pathogens for deaths associated with antimicrobial resistance, and the emergence of antibiotic-resistant strains such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for *S. aureus* has been approved [31].

### 2.67.1.2 *Escherichia coli*

*E. coli* or *Escherichia coli* is a rod-shaped bacteria that generally lives in the intestines of humans and most other mammals. Usually, it is harmless and quite beneficial for the health of the organism. However some variations of these bacteria are quite harmful and can cause severe food poisoning. Besides food poisoning, *E. coli* is also known to cause pneumonia as well as urinary tract infections, the latter of which can lead to serious health complications. Certain types of *E. coli* bacteria create a strong toxin called Shiga, which is one of the principal causes of human illness. The Shiga toxin can damage the lining of the intestine, which can eventually lead to a host of other illnesses. Consequently, the particular strains of *E. coli* that produce this toxin are called Shiga Toxin-producing *E. coli* (abbreviated as STEC) [31].

## 2.7 Subculture of test organisms

Subculturing is one of the microbiological techniques that are carried out to transfer the bacterial culture from one growth medium, such as broth or agar, to another and allow the microbes to grow. Subculture is used to prolong life and/or expand the number of cells or microorganisms in the culture. It is also useful in keeping strains alive by transferring them to a fresh growth medium.

The test organisms were subcultured as per the standard procedure to obtain pure cultures. An appropriate quantity of nutrient agar was dissolved in distilled water and autoclaved at 120 °C for 15 minutes at a pressure of 15 lb. Later, the nutrient medium was poured into the sterilized *petri* plates and allowed to solidify under aseptic conditions in a laminar airflow chamber. Bacterial suspensions of both the test organisms were prepared separately by adding 0.3–0.4 ml of nutrient broth to the lyophilized cultures. A loopful of bacterial suspension was streaked onto the solidified media in a zigzag fashion. The plates were incubated under aerobic conditions at 37 °C for 24 hours for *Staphylococcus aureus* and *Escherichia coli*, respectively.

### 2.7.1 Parallel Streak Method

The AATCC 147 method, commonly known as the Parallel Streak method, is designed to qualitatively evaluate the antibacterial activity of diffusible antimicrobial agents on treated textile materials. In other words, it tests the ability of the treated textile to inhibit the growth of microorganisms, i.e., to be bacteriostatic. This method permits the estimation of the activity and effectiveness of microorganisms at various concentrations.

#### 2.7.1.1 Preparation of Test Specimen

Test specimens (non-sterile) are cut by hand or with a die. They may be of any convenient size. Rectangular specimens cut at 25 x 50 mm are recommended. With a length of 50 mm, the

specimens can be spread across five parallel inoculum streaks that get narrower from 8 mm to 4 mm in width.

#### 2.7.1.2 Test Procedure

Dispense appropriate sterilized agar [cooled to  $47 \pm 2^\circ\text{C}$  ( $117 \pm 4^\circ\text{F}$ )] by pouring  $15 \pm 2$  mL into each standard (15 x 100 mm) flat-bottomed petri dish. Allow agar to gel firmly before inoculating. Prepare an inoculum by transferring  $1.0 \pm 0.1$  mL of a 24 h broth culture into  $9.0 \pm 0.1$  mL of sterile distilled water contained in a test tube or small flask. Mix well, using appropriate agitation. Using a 4 mm inoculating loop, load one loop full of the diluted inoculum and transfer it to the surface of the sterile agar plate by making five streaks approximately 60 mm in length, spaced 10 mm apart, covering the central area of a standard petri dish without refilling the loop. Take care not to break the surface of the agar while making the streaks. Gently press the test specimen transversely across the five inoculum streaks to ensure intimate contact with the agar surface. This may be accomplished more easily by pressing the specimen to the agar surface with a biological section lifter or with a spatula that has been sterilized by flaming and then air-cooled immediately before use [2]. Incubate at  $37 \pm 2^\circ\text{C}$  ( $99 \pm 4^\circ\text{F}$ ) for 18–24 h. The average width of a zone of inhibition along a streak on either side of the test specimen may be calculated using the following equation:

$$T - D$$

$$W = \frac{T - D}{2}$$

Where, W = Width of clear zone of inhibition (mm)

T = Total diameter of test specimen and clear zone (mm)

D = Diameter of test specimen (mm)

## 3. Results and discussion

### 3.1 Antimicrobial properties

Zinc oxide nanoparticles synthesized using *Citrus reticulata* peel aqueous extract were tested for their potential antimicrobial activity against selected microbes. The parallel streak method has shown that cotton fabrics treated with zinc oxide nanoparticles have shown a maximum inhibitory effect against *E. coli* and *S. aureus*. The results from Plate 1 (a) and (b) are indicative that ZnO-1 treated fabrics exhibit higher antibacterial activity in comparison with ZnO-2 treated fabrics, while the untreated fabrics exhibited no antibacterial activity. The antibacterial activity has been observed to be higher in the case of *E. coli* at 20.4 mm for ZAC2, followed by ZAC2 (18.5 mm), ZNC5 (15.8 mm) and ZNC6 (15 mm). While in *S. aureus*, the highest antibacterial activity was registered in ZNC6 (15 mm), followed by ZAC1 (14.5 mm), ZNC5 (14.5 mm) and ZAC2 (14 mm), whereas fabric treated with maleic acid showed a decrease in antibacterial activity. From Table 1, it is observed that fabrics treated with maleic acid as a cross-linking agent have shown a comparably lower zone of inhibition in both ZnO-1 and ZnO-2 treatments. From the Anova table, it is clear that there exists a significant difference between nanoparticles /nanoparticles and crosslinking agents in both bacteria.

The presence of ZnO NPs creates reactive oxygen species (ROS), specifically  $\text{H}_2\text{O}_2$ , OH; or  $\text{O}_2$ -radicals that interact with the cell membrane, causing oxygen to react with cysteinyl protein and iron inside the cells, thus leading to damage to the bacterial DNA and cell walls. The efficient antimicrobial activity of ZnO nanoparticles is explained as the generation of reactive surface oxygen, which kills bacteria by damaging the cell membrane of microbes [21]. Antibacterial activity is also influenced by many physiological characteristics of nanoparticles [1].

**Table 1. Antimicrobial properties of green ZnO nano-coated cotton fabrics**

Samples	Cross-linking agents	Antibacterial activity (Zone of Inhibition in mm)	
		<i>E. coli</i>	<i>S. aureus</i>
Control		NI	NI
<b>ZnO-1 precursor</b>			
ZAC1	Citric acid	18.5±0.46	14.5 ±0.48
ZAC2		20.4±2.33	14 ±0.92
ZAM3	Maleic acid	15.2±0.73	12.5±0.65
ZAM4		16±0.92	11.5 ±0.50
<b>ZnO-2 precursor</b>			
ZNC5	Citric acid	15.8±1.23	14.5±0.40
ZNC6		15±0.577	15 ±0.48
ZNM7	Maleic acid	13.7±1.03	11.3±1.41
ZNM8		12.7±0.77	13±1.01

NI – No Zone of Inhibition

Data are mean ± standard deviation of duplicate determination

\*ZnO-1: Zinc acetate dehydrate precursor

ZnO-2: Zinc nitrate hexahydrate precursor

#### ANOVA Table

C.D. (0.01)	Particulars	Antimicrobial properties	
		<i>E.coli</i>	<i>S.aureus</i>
	Nano particles (NPs)	59.47**	0.37
	Cross -Linking Agents (CLA)	12.25	6.51**
	NPs x CLA	12.89**	1.21
	C.V. %	8.14	11.68

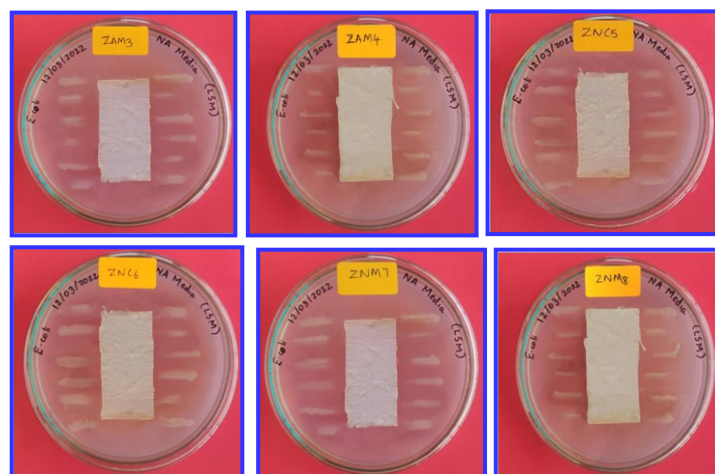
\*\*Highly significant @ 1 per cent level

### 3.2 Wash durability of green ZnO nano-coated cotton fabrics for Antibacterial activity

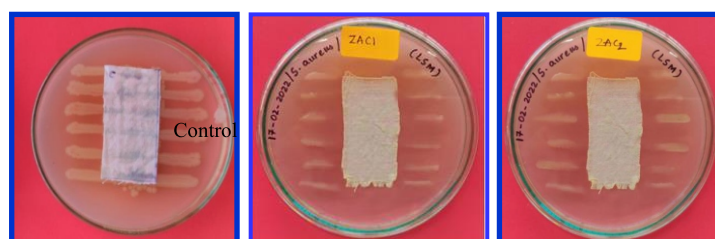
Wash durability tests were conducted for ZnO-NPs coated cotton fabrics and compared with the corresponding untreated fabrics. The results showed that the amount of coated ZnO NPs significantly decreased with the subsequent washes.

Irrespective of the bio-reduction agent, the pad-dry-cure method and crosslinking agents exhibited the maximum zone of inhibition before washing against *E. coli* (21.4±2.33) and *S. aureus* (15 ±0.48) as compared to the control sample. After the fifth wash, the zone of inhibition

was slightly decreased in both treated samples. All the samples treated with ZnO NPs retained their efficacy until all 5 wash cycles, with the highest ratings of 6.4±0.93 for (*E. coli*) and (8.4±0.94mm) for *S.aureus* bacteria. After five washes, there is a decline in the property. But it is worth noting that even after 10 washes, there is still antibacterial activity. Eventually, all the treated samples developed outstanding properties of antimicrobial activity (Table 2). From the t-test values, it is clear that there is a significant difference between the wash cycles and the treated fabric over the control (Fig. 1 and 2).



**Plate 1. a** Antimicrobial activity of synthesized green ZnO nano-coated cotton fabrics against *Escherichia coli* bacteria by Parallel streak method



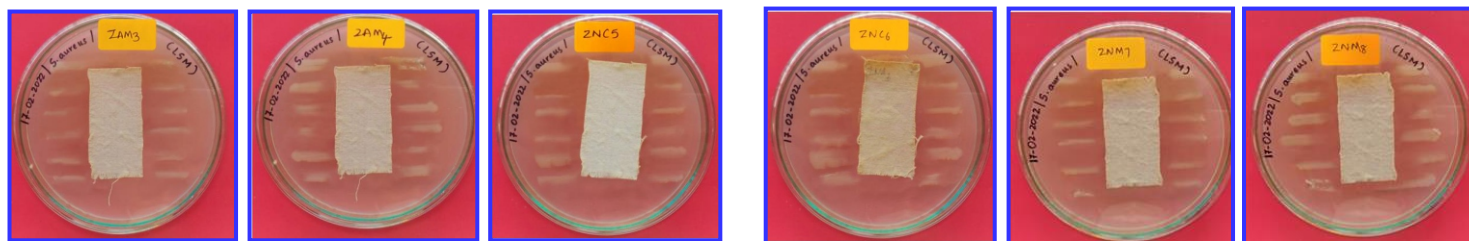


Plate 1. b Antimicrobial activity of synthesized green ZnO nano-coated cotton fabrics against *Staphylococcus aureus* bacteria by Parallel streak method

Table 2. Wash durability of green ZnO nano-coated cotton fabrics for Antibacterial activity

S. No	Samples	<i>E.coli</i> (Zone of inhibition in mm)				<i>S. aureus</i> (Zone of inhibition in mm)			
		0 wash	5 wash	10 wash	15 wash	0 wash	5 wash	10 wash	15 wash
1	Control	NI	NI	NI	NI	NI	NI	NI	NI
<b>ZnO-1 precursor</b>									
2	ZAC1	18.5±0.46	14±0.81	5.5±0.22	NI	14.5 ±0.48	7.5±1.47	3 ±0.35	NI
3	ZAC2	20.4±2.33	15.2±0.61	4.1±0.72	NI	14 ±0.92	8.2±0.81	3.1±0.69	NI
4	ZAM3	15.2±0.73	10.4±0.79	3 ±0.92	NI	12.5±0.65	6.5±0.81	2.5 ±0.40	NI
5	ZAM4	16±0.92	12.3 ±0.82	NI	NI	11.5 ±0.50	3.1±1.0	NI	NI
<b>ZnO-2 precursor</b>									
6	ZNC5	15.8±1.23	12.5±0.93	5.2±0.31	2.7 ±0.43	14.5±0.40	8.4±0.94	4±0.29	NI
7	ZNC6	15±0.577	11.5±0.81	6.4±0.93	3.4 ±0.71	15 ±0.48	5.2±0.81	2.3±0.33	NI
8	ZNM7	13.7±1.03	8.5±0.37	5.3±0.97	NI	11.3±1.41	4.2±0.81	NI	NI
9	ZNM8	12.7±0.77	6.2±0.66	NI	NI	13±1.01	5.2±1.47	NI	NI
	Mean	14.36	10.06	3.27	0.67	13.28	6.03	1.86	--
	Std. D	6.033	4.65	2.63	1.35	1.42	1.92	1.62	--
	T- test		11.09**	10.90**	12.64**		13.03**	27.4**	--

NI – No Zone of Inhibition

Data are mean ± standard deviation of duplicate determinations.

The wash durability test results showed a highly significant difference at a 1% significant level in numerous washings, according to the T-test results (Table 2). The proportion of bacterial reduction dropped as the wash cycle lengthened. The sluggish clearance of the extract may be due to the breakdown of cross-links between the finishing agent and the cellulose material, which may account for the decrease in antibacterial activity [8].

Similar results were obtained as per the ref. [14] with ZnO nano-coated cotton fabrics with antibacterial properties. According to the findings, the resulting cloth has substantial antibacterial action against *S. aureus* and *E. coli*. Also, gram-negative bacteria (*E. coli*) were more resistant to antibacterial treatments on unwashed and washed fabrics than gram-positive bacteria (*S. aureus*).

#### 4. Conclusion

The treated fabrics have direct contact of ZnO-NPs with cell walls, resulting in the destruction of bacterial cell integrity, the liberation of antimicrobial ions, mainly Zn<sup>2+</sup> ions, and ROS formation. ZnO-NPs have appealing antibacterial properties due to their increased specific surface area and reduced particle size, which results in higher particle surface reactivity. ZnO nanoparticle-coated cotton fabric can also be used in medical applications such as disposable bedding linen, antibacterial bandages, uniforms for health workers, etc. Advanced ZnO-functionalized cotton fabrics have a high potential for the preparation of advanced multifunctional textiles. Therefore, the rapidly advancing understanding of green synthesis outlined herein indicates that ZnO nanoparticles have immense potential for industrial production in the near future.

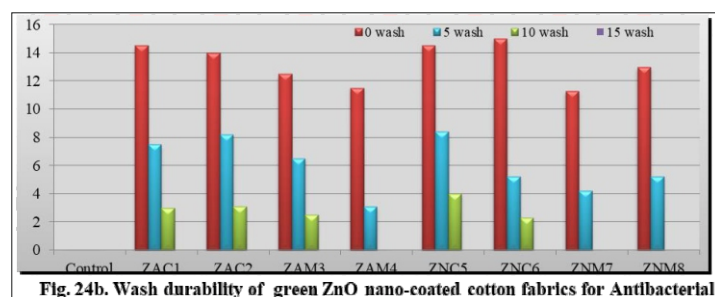
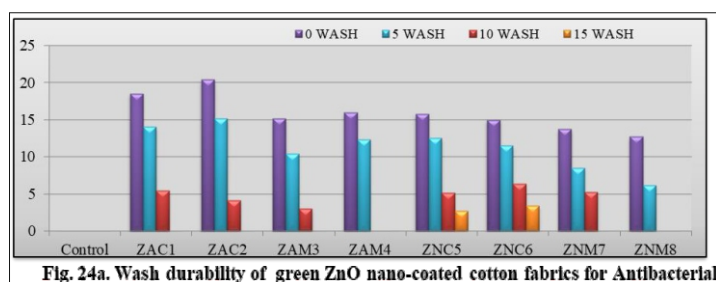


Fig. 2 Wash durability of green ZnO nano-coated cotton fabrics for antibacterial

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