FACULTY OF TEXTILE ENGINEERING TUL

Dissertation Thesis

Development of Cuprous Oxide based Antipathogenic Textiles

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ABSTRACT

The present work deals with the development of an environmentally friendly, low-price, easy, and fast method for developing antibacterial cuprous oxide-coated multifunctional cotton fabrics. At first, fabrics were sensitized with citric acid then the formation of Cu_2O particles was done by the Fehling solution method. Subsequently, the cuprous oxide particles were deposited on cotton fabrics. Three different types of reducing agents with different concentrations were selected to make the Cu_2O particles. Surface morphology and presence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. The antibacterial activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements. The strongest antibacterial effect was found for the fabrics coated with cuprous oxide particles reduced with sodium hydrosulphite. Furthermore, the utility of hygienic antimicrobial-developed fabrics was analyzed for comfort properties regarding air permeability and stiffness. In the end, the durability of the coating was confirmed by measuring the antibacterial properties and SEM analysis after washing.

In the second part of thesis a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with an excellent aesthetic appearance was developed**.** The objective of the second part was to develop an environmentally friendly, low-price, easy, and fast method for developing antipathogenic (antibacterial, antifungal, and antiviral) cuprous oxide-coated multifunctional fabrics. At first, fabrics were sensitized with citric acid then the formation of Cu_2O particles was done by the Fehling solution method. For sensitization and Cu_2O particles formation the same procedure was used. The most suitable reducing agent with optimum concentration was selected from the aforementioned study**.** Surface morphology and presence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. In the second step, a reactive antibacterial dye was made (by reacting Reactive Blue 4 with triclosan). The molecular structure of the modified dye was confirmed through FTIR and ^{13}C-NMR. The resultant antibacterial dye was applied on copper-treated cotton fabrics through exhaust dyeing protocol. The dyed fabric was characterized through colorimetric data (L*, a*, b*, C, H, and K/S), levelness of dye, fastness properties as well as exhaustion and fixation rates. The antipathogenic activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements. The strongest antipathogenic effect was found for the fabrics coated with cuprous oxide particles reduced with sodium hydrosulphite. Furthermore, the utility of hygienic antimicrobial developed fabrics were analysed for the comfort properties regarding air

permeability and stiffness. At the end, durability of coating was confirmed by measuring the antibacterial properties and SEM analysis after washing.
Keywords: antimicrobial; hospital-acquired infections; medical textiles; cuprous oxide particles; color analysis

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ABSTRAKT

Tato práce se zaměřuje na vývoj ekologického, nákladově efektivního, snadného a rychlého způsobu výroby antibakteriálních bavlněných tkanin potažených oxidem měďným. Nejprve byly tkaniny senzibilizovány pomocí kyseliny citrónové, poté následovala syntéza částic Cu_2O z Fehlingova roztoku. Následně byly částice oxidu měďného naneseny na bavlněné tkaniny. Pro výrobu částic Cu_2O byly vybrány tři různé typy redukčních činidel v různých koncentracích. Morfologie povrchu a přítomnost atomů kovů byly zkoumány pomocí rastrovací elektronové mikroskopie, dynamického rozptylu světla, FTIR, EDS a XRD. Antibakteriální účinnost tkanin potažených oxidem měďným byla hodnocena pomocí kvalitativních a kvantitativních analýz. Nejsilnější antibakteriální účinek byl pozorován u tkanin potažených částicemi oxidu měďného redukovanými pomocí hydrogensiřičitanu sodného. Dále byla analyzována vhodnost těchto vyvinutých hygienických, antimikrobiálních tkanin z hlediska komfortních vlastností, konkrétně prodyšnosti a tuhosti. Nakonec byla trvanlivost povlaku ověřena posouzením antibakteriálních vlastností a provedením SEM analýzy po praní.

Druhá část této práce představuje nový přístup k vytváření esteticky příjemných antibakteriálních bavlněných tkanin potažených oxidem měďným. Cílem této části bylo vyvinout ekologicky šetrnou, nákladově efektivní, snadnou a rychlou metodu výroby multifunkčních tkanin s antipatogenními vlastnostmi (antibakteriálními, antifungálními a antivirovými) potaženými oxidem měďným. Podobně jako v první části prošly tkaniny senzibilizací kyselinou citrónovou a syntézou částic Cu_2O pomocí Fehlingova roztoku. Na základě předchozí části práce bylo určeno nejvhodnější redukční činidlo s optimální koncentrací. Morfologie povrchu a přítomnost atomů kovu byla analyzována pomocí rastrovací elektronové mikroskopie, dynamického rozptylu světla, FTIR, EDS a XRD.

V následném kroku bylo syntetizováno reaktivní antibakteriální barvivo reakcí Reactive Blue 4 s triclosanem. Molekulární struktura modifikovaného barviva byla potvrzena pomocí FTIR a 13C-NMR. Toto antibakteriální barvivo bylo poté aplikováno na bavlněné tkaniny ošetřené mědí pomocí protokolu barvení vytahovacím postupem. Obarvená látka byla charakterizována pomocí kolorimetrických parametrů (L*, a*, b*, C, H a K/S), egality vybarvení, stálosti, vytažení z lázně a rychlosti fixace.

Antipatogenní účinnost tkanin potažených oxidem měďným byla hodnocena kvalitativně a kvantitativně. Nejvýraznější antipatogenní účinek vykazovaly tkaniny potažené částicemi oxidu měďného redukovanými pomocí hydrosiřičitanu sodného. Komfortní vlastnosti těchto hygienických, antimikrobiálně vyvinutých tkanin byly navíc hodnoceny z hlediska propustnosti vzduchu a tuhosti. Nakonec byla trvanlivost povlaku potvrzena vyhodnocením antibakteriálních vlastností a provedením SEM analýzy po umytí.

Klíčová slova: antimikrobiální; infekce získané v nemocnici; lékařské textilie; částice oxidu měďného; analýza barev

ABSTRAKT

Die vorliegende Arbeit konzentriert sich auf die Entwicklung einer umweltfreundlichen, kostengünstigen, einfachen und schnellen Methode zur Herstellung antibakterieller Baumwollstoffe, die mit Kupferoxid beschichtet sind. Zunächst wurden die Stoffe mit Zitronensäure sensibilisiert, anschließend erfolgte die Synthese von Cu2O-Partikeln mit der Fehling-Lösungsmethode. Anschließend wurden die Kupferoxidpartikel auf den Baumwollstoffen abgeschieden. Für die Herstellung von Cu2O-Partikeln wurden drei verschiedene Arten von Reduktionsmitteln mit unterschiedlichen Konzentrationen ausgewählt. Die Oberflächenmorphologie und das Vorhandensein von Metallen wurden mittels Rasterelektronenmikroskopie, dynamischer Lichtstreuung, FTIR, EDS und XRD untersucht. Die antibakterielle Wirksamkeit der mit Kupferoxid beschichteten Stoffe wurde durch qualitative und quantitative Messungen bewertet. Die stärkste antibakterielle Wirkung wurde bei Stoffen beobachtet, die mit Kupferoxidpartikeln beschichtet waren, die mit Natriumhydrosulfit reduziert wurden. Darüber hinaus wurde die Eignung dieser hygienischen, antimikrobiell entwickelten Stoffe hinsichtlich ihrer Komforteigenschaften, insbesondere Luftdurchlässigkeit und Steifigkeit, analysiert. Abschließend wurde die Haltbarkeit der Beschichtung durch die Bewertung der antibakteriellen Eigenschaften und die Durchführung einer REM-Analyse nach dem Waschen überprüft.

Im zweiten Teil dieser Arbeit wird ein neuartiger Ansatz zur Herstellung ästhetisch ansprechender, mit Kupferoxid beschichteter, antibakterieller Baumwollstoffe vorgestellt. Ziel dieses Abschnitts war die Entwicklung einer umweltfreundlichen, kostengünstigen, einfachen und schnellen Methode zur Herstellung multifunktionaler Stoffe mit antipathogenen Eigenschaften (antibakteriell, antimykotisch und antiviral), die mit Kupferoxid beschichtet sind. Ähnlich wie im ersten Teil wurden die Stoffe einer Sensibilisierung mit Zitronensäure und der Synthese von Cu2O-Partikeln nach der Fehling-Lösungsmethode unterzogen. Basierend auf der vorherigen Studie wurde das am besten geeignete Reduktionsmittel mit der optimalen Konzentration ermittelt. Oberflächenmorphologie und Metallpräsenz wurden mithilfe von Rasterelektronenmikroskopie, dynamischer Lichtstreuung, FTIR, EDS und XRD analysiert. Im darauffolgenden Schritt wurde ein reaktiver antibakterieller Farbstoff durch Reaktion von Reactive Blue 4 mit Triclosan synthetisiert. Die Molekülstruktur des modifizierten Farbstoffs

wurde mittels FTIR und 13C-NMR bestätigt. Dieser antibakterielle Farbstoff wurde dann über ein Ausziehfärbeprotokoll auf die mit Kupfer behandelten Baumwollstoffe aufgetragen. Der gefärbte Stoff wurde durch kolorimetrische Parameter (L*, a*, b*, C, H und K/S), Farbstoffegalität, Echtheitseigenschaften, Erschöpfung und Fixierraten charakterisiert. Die antipathogene Wirksamkeit der mit Kupferoxid beschichteten Gewebe wurde qualitativ und quantitativ beurteilt. Stoffe, die mit Kupferoxidpartikeln beschichtet waren, die mit Natriumhydrosulfit reduziert wurden, zeigten die stärkste antipathogene Wirkung. Darüber hinaus wurden die Komforteigenschaften dieser hygienischen, antimikrobiell entwickelten Stoffe hinsichtlich Luftdurchlässigkeit und Steifigkeit bewertet. Abschließend wurde die Haltbarkeit der Beschichtung durch die Bewertung der antibakteriellen Eigenschaften und die Durchführung einer REM-Analyse nach dem Waschen bestätigt.

Schlüsselwörter: antimikrobiell; im Krankenhaus erworbene Infektionen; medizinische Textilien; Kupferoxidpartikel; Farbanalyse

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"Seek knowledge from the cradle to the grave."

Prophet Muhammad (P.B.U.H)

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Muhammad Shahid, MSc.

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LIST OF ABBREVIATIONS

MIC	=	Minimum Inhibitory Concentration
ASTM	=	American Society for Testing and Materials
AATCC	=	American Association of Textile Chemists and Colorists
E. coli	=	Escherichia Coli
EDTA	=	Ethylenediaminetetraacetic Acid
EDX	=	Energy Dispersive X-ray Spectroscopy
EMG	=	Electromyography
EMI	=	Electromagnetic Interference
EPI	=	Ends per Inch
MRSA	=	Methicillin-resistant Staphylococcus aureus
NPs	=	Nanoparticles
PPI	=	Picks per Inch
SEM	=	Scanning Electron Microscopy
XRD	=	X-ray Diffractometry
HAIs	=	Hospital Acquired Infections
ICUs	=	Intense Care Unit
EIDs	=	Emerging Infectious Diseases
CNT	=	Carbon Nanotubes

1. INTRODUCTION

Hospital-acquired infections (HAIs) are on the rise despite efforts to decrease them. These infections develop in patients during their stay and continuous concern with the hospital. They are not only costly to treat, but more importantly, cause human suffering and even death. HAIs cause an estimated 100,000 deaths annually and account for up to $45 billion in health-care costs [1]. A major source of cross-infection is contaminated (bacteria and viruses) fabrics in hospitals. The most common textile coverage used in a specific hospital area (surgical, ICUs, and patient wards and rooms) included surgical gowns, drapes, curtains, panel covers, wall papers/sheets coverage, shoe mats, outlet covers, seat chair covers, table covers, patient and doctors' socks, etc. [2]. The patients, medical and common staff all are carriers of hospital-acquired infections [3]. The current interest has focused on high-touch textile-based surfaces and their ability to serve as reservoirs for pathogenic microorganisms, including *Staphylococcus Aureus*, *Clostridium difficile*, and *vancomycin-resistant Enterococci* [4, 5]. Instead of bacteria, among most pathogenies, the human infected viruses also contribute massively to hospital-born infections. Adding fuel to the fire, bacteria (after *Ethicillin* now *Methicelline*-resistant *Staphylococcus Aureus*) and viruses (a new strain called SARS-CoV-2) increasingly are becoming resistant to last-resort drugs. The rate of transmission of infection is very high and mainly spread during close contact and via respiratory droplets discharge and by touching a contaminated surface [6]. The risk can be increased when individuals have continuous and close contact with animals; also, climate change. The tragedy is that the infected person has been visiting the places and remain in touch with surfaces and people. It becomes too late when a person realized about infection and till that time infection has been delivered to many places, communities, or even to family members [7][8][9]. The only way to avoid life-threatening pathogens is to kill/inhibit them before transmitting them inside any human body. That is why the selection of antibacterial/antiviral common material used in daily life is necessary. A very quick and fast option to inactive the viruses and bacteria within a minute is the use of surface disinfectants with 62-71% alcohol, or bleaching agents containing 0.5% hydrogen peroxide and 0.1% sodium hypochlorite [9]. The alkali soaps (pH over 12) are working as well very efficiently. Hygiene standards for surface cleanliness, based on food processing industry standards also proposed [3]. Some new technologies, such as the use of UV light units and various hydrogen peroxide (HP) systems [10], can effectively decontaminate hospital rooms.

However, even when improved hand washing compliance and diligent surface hygiene disinfection are combined, hospital-acquired infections (HAIs) are still serious health issues. All the above approaches including hygiene hand washing, UV light, surface disinfection, and HP systems have one thing in common, they are episodic. or one-time approaches. Therefore, as soon as the decontamination process ends, the microbial contaminants can again begin to accumulate [11]. The researchers have been using different types of antimicrobial finishing on hospital textiles based on the coating of inorganic metal oxides [12][13]. The most common and particular interest among them is micro/nano particles of Ag, Cu, TiO_2, ZnO, MgO, CuO, Cu_2O, etc. [7][14]. They are not only stable under harsh process conditions but also generally regarded as odorless work wear. Copper-based materials are of most reliable because they are not only effective against microbes (within two hours) but are the only material that is most effective against the viability of pathogenic viruses (coronavirus within four hours). Copper, its ions, and alloys have demonstrated excellent antiviral, antibacterial, and antifungal activity against a wide range of pathogens [7][8][9]. The antibacterial properties of coper-based finishes are especially dependent on their shapes and sizes to assure a uniform size distribution of particles over the textile structure. In recent years, the copper and cuprous oxide particles have attracted so much attention in many potential applications in catalysis, cooling fluid or conductive inks, heat transfer systems, and antimicrobial, antifungal, and antiviral agents. Anita et al. [12] carried out a study to impart the copper oxide nanoparticles on fabric for achieving antimicrobial properties. The antibacterial property of the coated fabric was determined quantitatively and qualitatively using AATCC 100 and 147 test methods, respectively[12]. In another research Ali et al. [13] coated the copper oxide nanoparticles on cotton fabric to achieve antimicrobial properties. The copper oxide particles have very low stability and antimicrobial properties are affected. They can easily convert to different copper-based compounds like copper carbonate (greenish), copper sulphate CuS, etc. in different environment conditions. The resulting compounds formed by copper oxide particles are more toxic and cause acute poisoning some time[13]. Among copper-based (CuO, Cu, Cu_2O) anti-microbial agents the cuprous oxide particles are extremely regarded due to their optical, catalytic, mechanical, and, low-cost preparation. The cuprous oxides are more stable, effective, and beneficial regarding antibacterial properties. They are easily reducible and soluble in alkaline conditions. The antibacterial properties of copper-based finishes are especially dependent on their shapes and sizes to assure a uniform size distribution of particles over the textile structure. CuO and Cu_2O particles have been used to develop antimicrobial substrates. The CuO has various

limitations because of low stability, and can easily convert to different copper-based compounds like copper carbonate (greenish), copper sulphate, CuS, etc. in different environment conditions. The produced compounds are more toxic and cause acute poisoning. With the above background information work was focussed on the development of durable, stable, and evenly distributed cuprous oxide (Cu_2O) particles on the textile structure. In this developed study, we report a special technique to develop the Cu_2O particles[15]. Excellent antibacterial activities have been attained through the deposition of Cu_2O particles nanoparticles by employing these technologies. However, such treatments have led to some undesirable effects such as discoloration or staining on the coated fabrics which affect the aesthetic properties of textiles. In some studies, dyeing of the coated textiles has been performed to overcome discoloration and staining but their antibacterial effectiveness is compromised [16]. Thus, the development of highly effective antimicrobial textiles with improved aesthetics is yet challenging. Therefore, a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with excellent aesthetic appearance was investigated in detail. Considering the above-mentioned problems, the current study has proposed a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with an excellent aesthetic appearance. At first, fabrics were sensitized with citric acid then the formation of Cu_2O particles was done by the Fehling solution method. Then, the cuprous oxide particles were deposited on cotton fabrics. In the second step, a reactive dye was selected and functionalized as active against pathogens. The functionality was induced by reacting the Reactive Blue 4 dye with triclosan (antibacterial agent). Subsequently, the cuprous oxide particles coated fabric were subjected to exhaust dyeing through the solution of functional bioactive dye.

2. THESIS SIGNIFICANCE, SCOPE, AND OBJECTIVES

The main aim of the thesis is to investigate preparation properties and selected applications of bioactive textiles having the antimicrobial ability, antiviral, antifungal, and durability. To develop the copper-coated bioactive textiles a sufficient amount of copper ions is required over the fabric structure. This thesis is focused on the copper deposition on cotton fabric and subsequently dyeing it with antimicrobial dye (modified reactive blue 4 dye). The fabric used in this study was plain-woven cotton fabric with an areal density of "150 g/m^2."

At first, the formation of Cu_2O particles was done by the Fehling solution method and studied the effect of three different reducing agents to make the Cu_2O particles.

In the second step, the deposition of cuprous oxide particles was done on woven cotton fabric, and studied their antipathogenic (antibacterial, antifungal, and antiviral) properties.

The purpose of deposition of the cuprous particles over cotton fabric was to find a suitable application in hospital areas (surgical, ICUs, and patient wards and rooms) including surgical gowns, drapes, curtains, panel covers, wallpapers/sheets coverage, shoe mats, outlet covers, seat chair covers, table covers, patient and doctors' socks, etc [17][18].

The third step was to dye the already cuprous oxide-coated fabrics with antibacterial dye. In some studies, dyeing of the coated textiles has been performed to overcome discoloration and staining but their antibacterial effectiveness is compromised [19].

Thus, the development of highly effective antimicrobial textiles with improved aesthetics was challenging. The study has proposed a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with an excellent aesthetic appearance. At first, fabrics were sensitized with citric acid. Then, the Fehling solution method was followed for the synthesis of Cu_2O nanoparticles. The synthesized nanoparticles were then applied to cotton fabric. Then, a reactive dye was selected and functionalized with an antibacterial agent. Subsequently, the cuprous oxide particles coated fabric were subjected to exhaust dyeing through the solution of functional bioactive dye. The fabric treated with antibacterial dyes has poor washing durability due to which their application on fabric is not sustainable. Therefore, the synthesis and durable immobilization of nanoparticles particularly copper nanoparticles on textiles gained considerable attention in recent years due to their excellent washing durability. However, the textiles treated with copper or silver nanoparticles significantly alter the hue of the fabric thus affecting its aesthetic properties. Therefore, the

nanoparticles were applied on cotton fabric to achieve durable antimicrobial activity and the treated fabric was dyed with the an-bacterial dye to maintain its aesthetic as well as antibacterial properties.

The research activities were divided into the following three parts.

2.1 Formation of Cu_2O particles was done by the Fehling solution method

- The formation of Cu_2O particles by Fehling solution method.
- Study the effect of three different reducing agents to make the Cu_2O particles.
- Study the effect of different concentrations of developed Cu_2O particles on antimicrobial activity.

2.2 Deposition of cuprous oxide particles was done on woven cotton fabric and studied their antipathogenic (antibacterial, antifungal, and antiviral) properties.

- Study the effect of different concentrations of developed Cu_2O particles against antipathogenic (antibacterial, antifungal, and antiviral) properties
- Study the surface morphology of developed Cu_2O structures.
- Study the durability of Cu_2O coated textiles regarding washing and rubbing action.
- A developing process providing odorless work wear, less cost, and easy preparation.
- Study the comfort properties regarding air permeability and stiffness

2.3 Dyeing the already cuprous oxide-coated fabrics with antibacterial dye

- Synthesis of cuprous oxide nanoparticles by using three different reducing agents
- Application of the as-synthesized Cu-NPs on cotton fabric to impart antibacterial functionality
- Application of modified antibacterial dye on copper-coated fabric to improve aesthetic appearance.

So, we tried dyeing cuprous oxide-treated fabrics to make them suitable for commercial applications with enhanced antimicrobial ability. The end applications of the developed textiles are the fabrication of antimicrobial bed sheets, surgical drapes, panels, surgical gowns, pants, panel covers, wallpapers coverings, shoe mats, scrub suits, table coverings, chair coverings, socks for doctors and patients, etc.

3. LITERATURE REVIEW

3.1. Significance and Properties of bioactive textiles

Emerging Infectious Diseases (EIDs) are characterized by their emergence as newly identified or drug-resistant contagious illnesses. The incidence of EIDs has risen over the past two decades, with predictions indicating a potential increase in the future. EIDs encompass a variety of transmission channels and infection mechanisms; however, humans lack sufficient immunity and therapeutic options to combat these diseases. Regrettably, EIDs have presented an ongoing threat to human health and the environment for several decades. They first emerged with the Spanish flu in 1918, followed by the Asian influenza outbreak in 1957, and subsequently the Severe Acute Respiratory Syndrome (SARS) in 2003, the prevalence of Swine flu influenza in 2009, the Ebola outbreak in West Africa in 2014, the Zika virus outbreak in 2016, and most recently, the SARS-CoV-2 pandemic in 2019. In the past two decades alone, there have been documented instances of EIDs evolving into thirty different types, according to statistics [20]. EIDs pose a significant health challenge, leading to considerable economic losses and societal consequences due to their high fatality rate and rapid spread. For instance, the SARS pandemic resulted in 919 deaths, with 8,422 confirmed reported cases worldwide. The West African Ebola epidemic led to 11,323 fatalities and 28,646 reported cases in 2014. As of now, the cumulative count of confirmed cases of Coronavirus illness has exceeded 525 million, with 6.28 million deaths, causing negative per capita income growth in more than 95 percent of nations [21].

Bioactive textiles play a significant role in combating emerging infectious diseases. According to reports, the utilization of protective clothing, medical masks, gloves, and protective suits can effectively impede the growth and transmission of pathogens, thereby mitigating the rapid and deadly emergence of pandemic viruses. While protective clothing and masks contribute to slowing the transmission of the virus, it's important to note that complete prevention of infection cannot be guaranteed [22]. The outbreak of SARS-CoV-2 has heightened the demand for bioactive textiles, and their importance is expected to further escalate in the post-pandemic period as a crucial means of safeguarding humanity.

Microbes present in our surroundings encompass bacteria, fungi, algae, and viruses. Among these, nearly 80% of human diseases are transmitted and propagated through textile surfaces that have been contaminated by these microbes. Moreover, microbes residing within the fibers can give rise to cross-contamination, fading of colors, and undesirable odors in textiles. To counteract these adverse effects, antimicrobial properties are incorporated into

textile materials, resulting in the development of bioactive textiles. Bioactive textiles exhibit antimicrobial characteristics effective against a range of microorganisms, including bacteria, fungi, and viruses. A brief overview of these properties is presented below.

3.2. Antibacterial properties

Bacteria are minuscule unicellular organisms found in millions in all environments, both outside and within other organisms. These pathogens are human allergens, stimulators, environmental toxins, disease-causing agents, and just unpleasant substances. Bacteria are dangerous pathogens in the home, on clothing, and in furnishings. Moisture, water, nutrients, and most crucially, a surface are all necessities for bacteria to grow [23]. Bacterial cells can get nourishment from dust, soil, and various textile finishes. Salts, carboxylic acids, amino acids, and other vital elements are found in human sweat. Deteriorated cellulose from textiles, as well as dead skin or oils released by the body's skin, are rich sources of nutrition for bacterial growth [24] Various bacteria contact the human body through contaminated textile materials on the skin and cause a variety of diseases in humans. *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, are among the bacteria that commonly contaminate the surface of textiles. These bacteria can have harmful effects on humans because of user contact and cross-infection [25]. To address these issues, textiles are treated with various antibacterial agents to impart antibacterial functionality to them. Antibacterial textiles can shield the textile from bacterial attack, limit the transmission and proliferation of pathogenic bacteria, as well as reduce odor caused by bacterial deterioration. To get the most out of antibacterial textiles, the ideal antimicrobial treatments must meet several criteria such as low cost, eco-friendly, and non-toxic to humans. The antibacterial textiles can kill (bactericidal) or inhibit (bacteriostatic) the growth of bacteria [25].

3.3 Antiviral properties

Viruses are a unique form of matter that lies between non-living and living entities, primarily composed of protein and genetic material. Viruses are categorized as either enveloped or non-enveloped based on their external structure. Non-enveloped viruses are encapsulated by capsid proteins, rendering them resilient to high temperatures and acidic conditions, thus making it challenging to inhibit their growth in vitro. On the other hand, enveloped viruses possess a lipid membrane in addition to a protein shell, allowing them to adapt to diverse physiological environments and extend their duration within a host.

However, this lipid membrane is susceptible to high temperatures and acidic conditions, making it easier to disrupt virus growth in vitro.

Viruses have the capability to survive on various surfaces. Research indicates that viruses can persist on textile materials for 1 to 4 weeks at room temperature, potentially leading to cross-infection and re-infection [26]. Viruses can also be transmitted through aerosols and droplets, facilitating disease spread. The use of antiviral textiles (personal protective equipment) and household fabric masks can help control virus transmission.

Antiviral Textile materials can kill viruses present on the surface of textiles or prevent biofilm formation, lowering the infection risk and re-infection. Due to the constant emergence of novel contagious diseases, antiviral textiles research has garnered considerable attention. Antiviral fabrics can effectively prevent viruses from spreading and limit the cross-infection risk. Researchers have been studying several antiviral materials in recent years to see if they may stop viruses from spreading and reproducing by killing them or limiting their attachment. Finishing and other spinning procedures can be used to incorporate these elements into antiviral fabrics. Actively damaging the viral structures, interacting with viral adsorption, prohibiting the virus from infiltrating cells, suppressing virus synthesis, restricting the release of the virus, or improving the antiviral capability of the host are the most common antiviral mechanisms [27].

3.4. Antifungal properties

Fungi, microscopic organisms, can be found in both outdoor and indoor environments. A prior study has identified correlations between exposure to fungi in the environment and its impact on human health. The prevalence of asthma and increased asthma-related mortality have been associated with fungal exposure. Fungal exposure is also linked to various disorders, including allergic fungal sinusitis, allergic bronchopulmonary mycosis, hypersensitivity pneumonitis, as well as asthma and rhinitis. Common outdoor fungi include *Alternaria, Cladosporium, basidiospores,* and *ascospores* [28]. However, due to their ability to infiltrate through windows and open doors and subsequently be transported indoors, these fungal species are often found indoors as well. Notably, two textile scientists from Cornell University have reported that clothing, primarily made of cellulose, worn by patients and visitors in hospitals serves as a significant source of *Aspergillus* fungal spore transmission [29]. Aspergillus represents a group of mold fungi that are commonly present in environmental conditions worldwide and are regularly inhaled. This ubiquitous fungus poses a potentially fatal infection risk, particularly for healthcare workers with compromised

immune responses. Interestingly, cotton has been found to carry and disperse *Aspergillus* spores more than any other studied material.

Antifungal textile materials can safeguard textiles against fungal attacks and substantially mitigate the risk of fungal transmission. The suggested mechanism for antifungal textiles involves damaging cell walls, hindering cell wall biosynthesis, suppressing enzymatic activity, and restricting nucleic acid and protein synthesis. The implementation of antifungal properties is often achieved through finishing processes. A variety of antifungal agents are commonly employed during the finishing process, including quaternary ammonium compounds, triclosan, polyhexamethylene biguanide (PHMB), and silver [29].

3.5. Antimicrobial Agents for bioactive textiles

The term 'antimicrobial' encompasses a broad array of strategies that offer varying degrees of protection to textile fabrics against microbes. The chemical composition, mode of action, impact on the environment and humans, handling characteristics, durability, regulatory compliance, costs, and the interactions with microbes can all differ significantly [30]. Antimicrobial agents serve to either exterminate microorganisms or hinder their growth. Agents referred to as biostats, bacteriostats, or fungistats, for instance, restrict microbial growth without inducing death. Conversely, biocides, encompassing bactericides and fungicides, bring about the death of microorganisms. Antimicrobial compounds are employed within the textile industry to impede the growth of microorganisms.

Over the years, researchers have identified numerous antimicrobial agents capable of eradicating microbes through diverse mechanisms. Presently, there is no standardized classification for antimicrobial agents. Commonly used antimicrobial agents are generally categorized into three groups based on their chemical structure: organic, inorganic, and natural. A brief overview of these categories is provided here. The primary types of antimicrobial agents, along with their advantages and disadvantages, are summarized in Table 1 [30].

3.5.1. Inorganic antimicrobial agents

Inorganic antimicrobial agents represent an emerging category of bioactive materials, encompassing both carbon-based substances and those containing metallic ions. Metal nanoparticles possess distinctive attributes including a high specific surface area, strong stability, excellent biocompatibility, low toxicity, and ease of functionalization, which collectively contribute to their extensive use in various biomedical applications [31].

Commonly employed metallic nanomaterials include silver, copper, titanium, and gold nanoparticles. Silver nanoparticles (AgNPs) have gained significant attention due to their broad-spectrum antimicrobial properties, safety profile, absence of antibiotic resistance mutation, and cost-effectiveness. They have been extensively utilized for imparting antimicrobial properties to textiles [31]. Silver nanoparticles have demonstrated efficacy against a range of viruses including hepatitis B, monkey pox virus, and distemper viruses, as well as bacterial species like *S. aureus and E. coli* [32]. Copper (Cu), another prevalent antimicrobial substance, is favored for its high efficiency, low toxicity, and broad-spectrum action. It can combat infectious bronchitis viruses, poliovirus, HIV-1, *S. aureus, E. coli*, and other pathogens. Titanium dioxide (TiO_2), a photocatalytic nanomaterial, exhibits antimicrobial potential against viruses and bacteria by utilizing photocatalysis. Gold nanoparticles (AuNPs), owing to their unique properties stemming from their small size effect, surface effects, and macroscopic quantum tunneling effect, are gaining attention for bioactive textiles and are also frequently used as carriers for antiviral drugs [33]. The antimicrobial mechanism of these metallic nanoparticles involves disrupting protein structures, impeding DNA replication, and deactivating essential microbial enzymes, ultimately leading to microbial cell death.

Carbon-based nanostructures encompass carbon materials with at least one dimension less than 100nm in the dispersed phase. Such structures possess inherent antimicrobial properties and can leverage their exceptional electrothermal and electrochemical capabilities for microbe inactivation through heating. Additionally, their nanostructures might disrupt viral reproduction. Emerging carbon-based nanomaterials such as graphene and carbon nanotubes (CNTs) exhibit antimicrobial properties. These materials are constructed from carbon atoms arranged in a sp^2 hybridized honeycomb hexagonal lattice, boasting remarkable mechanical, optical, and electrical characteristics. The application of these nanostructures to inhibit virus growth via electric and photoheating mechanisms is promising, given that viruses are unable to withstand elevated temperatures [34].

3.5.2. Organic antimicrobial agents

Organic antimicrobial agents offer advantages such as rapid disinfection, straightforward synthesis procedures, and high effectiveness. These agents find application in the development of antimicrobial textile materials through textile finishing processes. Organic antimicrobial agents can be cross-linked and copolymerized with other comonomers to create antimicrobial coatings and fibers. Examples of such agents include organic metals,

quaternary ammonium compounds, organic heterocyclic compounds, and phenolic compounds. Quaternary ammonium compounds, particularly quaternary ammonium salts, are among the most widely used. The antibacterial activity of these compounds is influenced by their structure, particularly the lengths of the alkyl chains. Double-chain quaternary ammonium salts, for instance, can inactivate microbes effectively [34].

Organic heterocyclic bioactive agents, particularly those containing nitrogen, such as pyrrole, pyrazoles, and pyrimidines, have long been employed as antimicrobial agents for producing antimicrobial textiles [35]. Apart from the aforementioned categories, organic antimicrobial materials encompass organotin compounds, biguanides, and various other compounds. The mode of action of organic antimicrobial agents involves both physical and chemical interactions. Positively charged organic active agents can interact with negatively charged bacterial cell walls through electrostatic interactions. Alternatively, antibacterial agents can penetrate bacterial cells, obstructing the synthesis of crucial proteins and nucleic acids while also inhibiting metabolic pathways, ultimately leading to microbial death [36].

3.5.3. Natural antimicrobial agents

Natural antimicrobial agents are primarily obtained from plants as well as active compounds with antimicrobial capabilities, which are acquired by extracting, separating, and then purifying the extracted substances. Natural antimicrobial agents have received increasing attention in recent years because of increased ecological sustainability, health, and safety concerns. Natural antimicrobial compounds offer several benefits, including widespread availability, non-toxicity, and safety. Animals, plants, and microbial extracts are the most commonly employed natural antimicrobial components [37]. A significant portion of natural antimicrobial agents is sourced from plants. Plants possess an almost boundless capacity to generate aromatic compounds, many of which are phenolic compounds or their oxygen-substituted derivatives. These compounds function as a defense mechanism for plants, safeguarding them against microorganisms [38]. The textile industry has also incorporated natural colorants for their antimicrobial properties. Natural colorants such as pomegranate, lawsone, turmeric, juglone, curcumin, lapachol, henna, quercus infectoria, as well as aloe vera, eucalyptus oil, neem extract, tulsi, tea tree oil, silk sericin, clove, and onion extract, among others, serve as naturally occurring antimicrobial agents. Other widely employed plants for conferring antimicrobial attributes to textiles include acacia, gallnut, walnut, basil, arnebia nobilis, barberry, madhuca indica, rhubarb, ratanjoti, ashoka, gromwell, and peony [39].

Table 1: Types, advantages, and disadvantages of antimicrobial agents [30].

Antiviral material	Advantage	Disadvantage
Inorganic antiviral material Metal and their compound (e.g., Cu, Ag nanoparticles)	broad spectrum, Long term antiviral action, Low toxicity	High cost Delayed effect Ease of oxidation
Carbon Based Nonmaterial Photocatalytic material (graphene, carbon nanotubes etc.)	Low cytoxicity Specific antiviral activity Good antiviral action Eco-friendly	High cost Limited range use Poor antifungal action
Organic antiviral material (e.g. halogenated phenol)	Low cost Fast germicidal speed Easy processing Good Color stability	Easy to develop drug resistance Toxic Poor heat resistance
Natural antiviral material (Neem, Aloe vera extract etc.)	Safe and Nontoxic Eco-friendly	Difficulty in processing Poor durability Short life span Narrow application range
Composite antiviral material (Ag-chitosan composite)	Prolonged durability Broad spectrum Safe	The coordination mechanism is not clear

3.6. Comparative performance of copper and its oxides

Copper, a well-known noble metal, displays a distinctive bright colour and is found in its elemental state within the Earth's crust. Possessing qualities of softness, ductility, and strong corrosion resistance. The copper nanowires and particles that are commonly distributed both on and beneath the Earth's surface. Notably, the pliability and malleability of materials remain unaffected even after the application of nano-coatings comprising these metals. Copper ranks among the most commonly found and easily accessible metals post smelting processes. Following aluminum and iron, it stands as the third most significant commercial metal [40]. Often referred to as the "red metal," copper boasts a density of 8.94 g/cm³, an atomic weight of 63.57, and an atomic number of 29. It exists in two valence forms: +1 (cuprous) and +2 (cupric), with +2 (cupric) being predominant. In its naturally occurring isotopic forms, copper has mass numbers of 65 (31%) and 63 (69%). Generally, cuprous salts undergo auto-oxidation to generate cupric salts, as depicted in Equation 1.

$$2\,Cu^{+} \rightarrow Cu + Cu^{++} \qquad (1)$$

Copper exhibits a broad spectrum of antimicrobial activity, rendering it effective against a wide range of bacterial strains, encompassing both Gram-negative and Gram-positive bacteria. Its bactericidal effects are evident across various levels of humidity and temperature, underlining the significance of copper coatings as antibacterial agents, even within the typical temperature of a hospital room. Copper has the ability to permeate the skin of both animals and humans, leading to microbial elimination. Experiments were conducted to evaluate the potential inflammatory responses induced by this therapeutic impact [40][41].

In another experiment, bis(glycinato) copper, a compound identical to skin (II), was utilized to examine its effects on the skin. Liu et al. (2010) conducted a similar experiment on the skin of a cat to assess copper's antimicrobial activity. Electron micrograph images of the treated skin revealed the impact of copper exposure. Additionally, atomic absorption spectrometer results indicated a copper content of 47 ppm on the cat's skin [41][42].

3.7. Necessity of antimicrobial agents

Microbes are also abundant in the human body, with approximately 100-1000 microbes/cm² present on clean skin. The skin harbors a diverse range of microbes, including fungi, bacteria, and yeast, which can potentially infect the wearer. These disease-causing microbes exert various effects on the human body, including the development of foul odors and the discoloration of textiles due to staining. Microbial growth on textiles can lead to a multitude of issues not only for the fabric itself but also for the individual wearing it. Textiles provide an effective medium for infection-causing microbial species to adhere, transmit, and multiply, facilitated by the properties of textiles that make them susceptible to interaction with the human body [43]. Additionally, textile characteristics such as porosity and the hydrophilic nature of natural fibers render them more susceptible to microbial attacks compared to synthetic fibers. Natural fibers serve as an ideal nutrient-rich environment for rapid microbial proliferation, as they can retain nutrients, water, and oxygen. Coupled with the warmth, nutrients, and humidity originating from the human body, these conditions create an optimal setting for microbes to rapidly grow and multiply. The most harmful pathogens that grow on the skin include fungi and bacteria. Conversely, algae can thrive on textiles that remain damp for extended periods. Fungal growth on fabrics can lead to discoloration, fabric damage, and staining. Additionally, it can cause unpleasant odors and a sticky, slimy sensation upon contact. Microbial growth on textiles can also be influenced by the structure and chemical composition of the textile materials themselves [44]. This issue is challenging to resolve due to the prevailing warmth and humidity in the fabric's surroundings.. In contrast

to natural materials, synthetic materials do not offer favorable conditions for microbial proliferation. This is primarily due to the absence of nutrients, and it may also be attributed to the use of low molecular weight contaminants in textile finishing processes. These microbes can give rise to undesirable odors, fabric staining, and even contribute to a decrease in textile strength. In order to minimize microbial growth on textiles during use and storage, several factors must be considered [45].

3.8. Interaction of microbes with textile fibers

Microbial growth has notably distinct effects on natural and synthetic fibers. Both natural and synthetic fibers can serve as suitable substrates for microbial growth and colonization, yet the underlying mechanisms of their growth on these fabrics are inherently different. In the case of natural fibers, microbial enzymes can readily hydrolyze the polymer bonds within them, facilitated by the ample presence of moisture. As a result, natural fibers are particularly susceptible to microbial degradation. Notably, a wide range of cellulosic and protein fibers can be easily targeted by microbes. Multiple reports from various sources have indicated that cotton, jute, wool, flax, and silk are among the most commonly attacked fibers susceptible to microbial infestation [46].

Microbes tend to grow at a slower pace on synthetic fibers in comparison to natural fibers, primarily due to the polymeric structure of synthetic fibers that restricts water absorption. Conversely, these fibers tend to accumulate sweat within their intercellular spaces, providing an environment conducive to rapid microbial proliferation. Notably, it has been observed through various studies that socks crafted from synthetic fibers are more susceptible to foot infections than those fashioned from natural fibers. Earlier research indicates that bacterial adherence to fabric increases as the polyester content in the textile rises. Additionally, if synthetic material processing involves finishing treatments such as polysiloxane and polyethylene emulsions, the susceptibility of synthetic fibers to microbial degradation escalates. These substances provide microorganisms the means to break down the polymer structure into smaller, more manageable components, facilitated by the production of basic or acidic metabolic by-products, thereby initiating a hydrolysis cycle. Even robust synthetic polyurethanes can be degraded through the same microbial pathway. Under conducive conditions, the impact of microbes on different synthetic fibers such as polyester, polypropylene, and nylon has been documented [47].

Textiles not only serve as substrates for bacterial growth, but they can also actively contribute to the proliferation of microorganisms, which presents a more significant concern.

The survival of viruses on various fabrics, including washable wool suits, terry towels, cotton shirting, polyester/cotton shirting, and nylon jersey, can extend up to 16 hours. Viruses tend to persist and propagate more effectively on synthetic fibers compared to natural materials. While laundering can physically remove viruses from fabric, their complete inactivation does not necessarily occur. Instead, they are detected and washed away with the extracted laundry water. Detergents play a role in lowering surface tension, aiding in the physical removal of viruses. Moreover, certain bacteria can endure on fabrics even after undergoing washing.

The presence and proliferation of microorganisms on textiles can lead to health concerns, alterations in fabric odor, and eventual fabric deterioration. Microbial attacks can result in staining and the loss of functional attributes such as tensile strength and elasticity, which can also arise due to the usage of additives in textiles. As microorganisms proliferate, they metabolize available nutrients from the environment, such as perspiration and soiling, producing odor-causing chemicals. For instance, the Gram-positive bacterium *S. aureus* is believed to generate 3-methyl-2-hexanoic acid, responsible for the distinctive body odor. The production of malodor stands as one of the most prominent negative consequences of microbial growth on fabric [48]. Among other factors, bacteria have the capacity to transform human perspiration into odorous compounds including amines, carboxylic acids, and aldehydes, contributing to unpleasant odors. In the case of a Gram-negative bacterium like P. Vulgaris, the metabolism of urea can lead to the generation of ammonia, causing odor issues, as observed in infant diapers [48].

3.9. Interaction of antimicrobial agents with microbes

Antimicrobial drugs interact with microbes in two ways, they either kill microorganisms or hinder their growth [49]. The mode of action of antimicrobial agents on microbes is either damaging the cell-wall of microbes, restricted cell-wall synthesis, altered cell-wall permeability, inhibiting the metabolic processes, interacting with the plasma membrane, inhibiting the essential protein synthesis, restricting the nucleic acid replication and transcription and inhibiting the enzymatic activity of the microbial cell.

There are several antimicrobial compounds used in textiles that had previously been studied in the food and cosmetics fields. These compounds are absorbed into textile substrates in small quantities than other chemicals. While using these compounds it is necessary to make sure that these compounds are not only persistently efficacious but also safe to use on the human body as well as in an external environment. Active finishes are those materials that contain effective antibacterial chemicals that might act on the genome of

pathogenic cells or might interrupt the metabolic processes of microbes. These antimicrobial products have unique nature in their action, it is not easy to distinguish between antimicrobials and other active compounds due to the wide range of applications [45]. Antimicrobial compounds come in a variety of forms and they have their mechanism of action. A few impacts of antibacterial drugs are acting as oxidizing agents which include peroxy compounds, aldehydes, and halogens. These compounds normally target the cell membrane they usually enter the cytoplasm and disrupt the essential enzymes of microbes which results in the killing of microbe. Some other antimicrobials are coagulants which are typically alcohols that irreversibly deform protein molecules. Peroxy compounds such as halogens, and isothiazones are extremely reactive due to the availability of free electrons. These chemicals react mostly with all organic materials particularly oxidizing thiols in amino acids of the microbial cell. These chemicals are extremely harmful to nucleic acids even if available in very small quantities [50].

Another type of antimicrobials is diphenyl ether (bisphenyl) compounds which are also known as 5-chloro-9 2-(2, 4-dichloro phenoxyl) phenol or 2, 4, 4'-trichloro-2' hydroxy dipenyl ether. These are highly durable antibacterial compounds. Triclosan has been used for a long time around 25 years in personal hygiene and hospital products which includes antibacterial soaps, mouthwash, and antiperspirants. Triclosan inhibits microorganism proliferation by penetrating and disrupting an electrochemical mechanism of their cell walls. When cell walls of microbial cell rupture metabolites present inside the cell leach out which results in impairing its cell activities are ultimately the organism will unable to operate or reproduce. When Triclosan forms a polymer, it starts to move to the surface and form a bonded there. As it is not soluble in water so it is not leached out and it is used as a blocking or barrier action. Finally, it permanently limits the growth of pathogens making contact with the substrates [50]. Some microbe inhibitors are chemicals that bind to the microbial cell membranes and lead to cell decomposition by disrupting the lipo-polysaccharide backbone. These are poly cationic, absorbent having porous qualities such as amines, quaternary ammonium compounds, glucoprotamine, and biguanides. The active enzyme centers of the microbial cell can also inhibit by using metals such as mercury, cadmium, copper, and silver. These metals are responsible for inactivating the active enzymatic centers (inhibition of metabolic processes). For example, silver complexes have already been used as an antibacterial in the formulation of drinking water [50]. Chitosan is a natural antibacterial agent which is found in crustacean shells. Chitosan-coated conventional fibers are a more suitable antimicrobial agent as it does not trigger an immune response and these fibers are

easily available in the market. Other natural herbal materials used in the antimicrobial finishing of textiles are a wide variety of herbal medicines having antimicrobial properties that can also be used to make herbal textiles. Natural bioactive compounds with antimicrobial activities have been used for biofunctionalization of textiles. These compounds enable the development of bioactive textile products and they are non-toxic, safe, and eco-sustainable. Some bioactive components are extracted primarily from plants include phenolics and polyphenols (quinines, simple phenols, tannins, phenolic acids, flavonoids, flavonols, coumarins, and flavones), lectins, terpenoids, essential oils, alkaloids, polypeptides, and polyacetylenes. Many compounds are employed as natural antimicrobial pigments and dyes for dyeing synthetic and natural fabrics and many of them are recognized as colored chemicals [51][52]. Microbes including fungi and bacteria have also been used to make ecologically sustainable colors [53][54].

Metallic nanoparticles have antibacterial properties which might be due to their large specific surface area and nano-scale particle size. Nanoparticles have a higher specific surface area through which they interact with microorganisms and by decreasing the particle size their antimicrobial activity increases dramatically [55]. Due to having the huge specific surface area of nano particles that allows for a significant rise in the concentration of metallic cations emitted from the surface of the particle. These metallic cations have direct antibacterial action which results in greater biocidal activity. The major harmful effect of nanoparticles on microbial cells is oxidative stress, which causes damage to DNA, proteins, and lipids [56], which causes damage to DNA, lipids, and proteins of pathogens. Higher solubility of metallic oxide nanoparticles has also been shown to improve their antibacterial efficacy [57], suggesting that the increase in the concentration of metallic ions emitted from the surface of the nanoparticles has a significant impact on their action and toxicity. The photo-catalytic destruction of bacteria by TiO_2 gives it antimicrobial characteristics [58][59]. The toxicity of nanoparticles and metallic is induced by their slow release from the surface, according to the suggested antimicrobial mechanism; this is also true for textile materials treated with noble metals and metallic oxide nanoparticles. When compared to extremely biocidal nanoparticles, micron-sized metals did not cause cell harm [47]. Figure 1 depicts a review of the mechanisms involved in metallic nanoparticle's antibacterial activity.

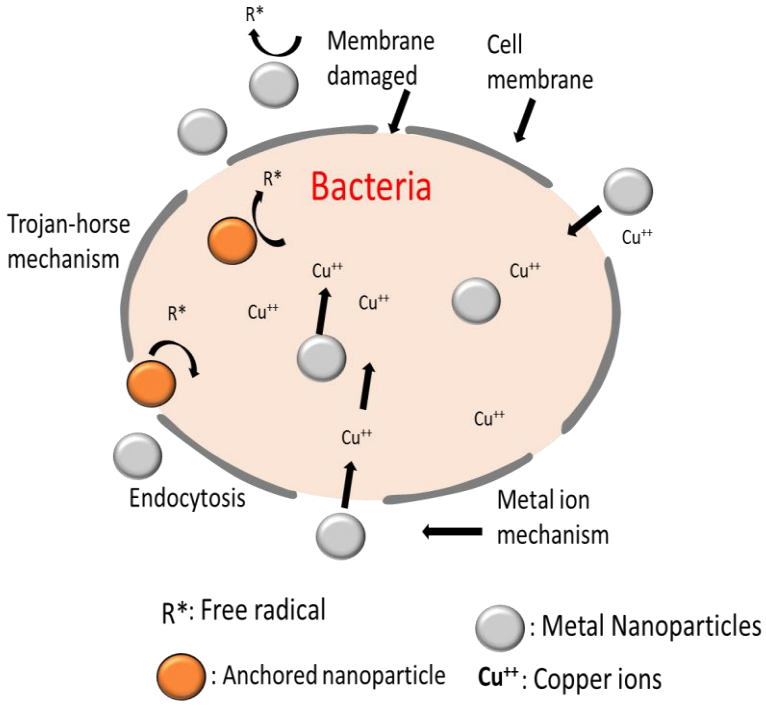


Figure 1: A brief explanation of the processes underlying the antibacterial action of metallic nanoparticles[47].

The comparable size of nanoparticles and biomolecules including proteins and polynucleic acids contributes to their antibacterial effects. Chemical interactions, physical interactions, or a combination of both may be responsible for nanoparticles antibacterial properties.

3.9.1. Chemical interactions

Chemical interactions of Cu^{++} ions can occur in the interior of the cell and the first step is the transportation of Cu^{++} ions across the membrane. Constituents of the cell membrane and H_2O_2 react with copper and start the antimicrobial process. Organic complexes can be formed when Cu nanoparticles react with oxygen, sulphur-, or nitrogen -containing functional groups in the microbe. These particles can also cause alteration in oxidative phosphorylation and osmoregulation as well as abnormalities in the structural changes of proteins and nucleic acids. When microbes are exposed to hazardous levels of nanoparticles they up-regulate genetic makeup and finally cause the removal of reactive oxygen species leading to oxidative stress to microbial cells [60].

3.9.2. Physical interactions

In terms of physical interactions, the transport mechanism of cellular membranes is disrupted by copper nanoparticles. The abrasive effect of copper nanoparticles on cellular components results in physical degradation, ultimately facilitating the penetration of copper

nanoparticles through cellular membranes. This interaction extends to the inner membrane, initiating an immediate electrostatic contact between copper nanoparticles and the constituents of the bacterial cell membrane [60].

3.9.3. Combination of the physical and chemical interaction

The antimicrobial action of copper nanoparticles results from a combination of both physical and chemical interactions with microbes. The aforementioned discussion underscores the intricate nature of the antimicrobial properties exhibited by copper nanoparticles. Notably, both the nanoparticles themselves and their ions can participate in biocidal processes, with metallic nanoparticles standing as the active and potent antimicrobial agents.

Copper is an essential element for the survival of all types of cells and is required in appropriate quantities. Consequently, cells possess copper homeostatic mechanisms to maintain safe internal copper levels. However, external copper exposure can disrupt this homeostatic balance, leading to an overload of intracellular copper under diverse environmental conditions. This imbalance proves detrimental to microbial cells. The toxicity induced by copper manifests through various pathways, and identifying the exact mechanism active against specific bacteria is complex. The most prevalent and well-established mechanism involves inducing lethal oxidative damage to cells through redox reactions. Additionally, ongoing research continues to explore various other mechanisms, highlighting that copper's impact on cells varies based on growth conditions and the surrounding environment [61].

Copper's entry into cells occurs through routes that are not yet fully understood. Within the cell cytoplasm, copper has the potential to convert to Cu+, leading to the production of highly reactive hydroxyl radicals. Subsequently, nonspecific reactions ensue with nucleic acids, lipids, free amino acids, and proteins. In anaerobic environments, copper-glutathione compounds may form. Alternatively, copper ion and iron react, resulting in Cu+ displacing iron from iron-sulfur complex proteins, a widely recognized mechanism of toxicity. The toxicity mechanism associated with copper nanoparticles is depicted in Figure 2 below [62][63].

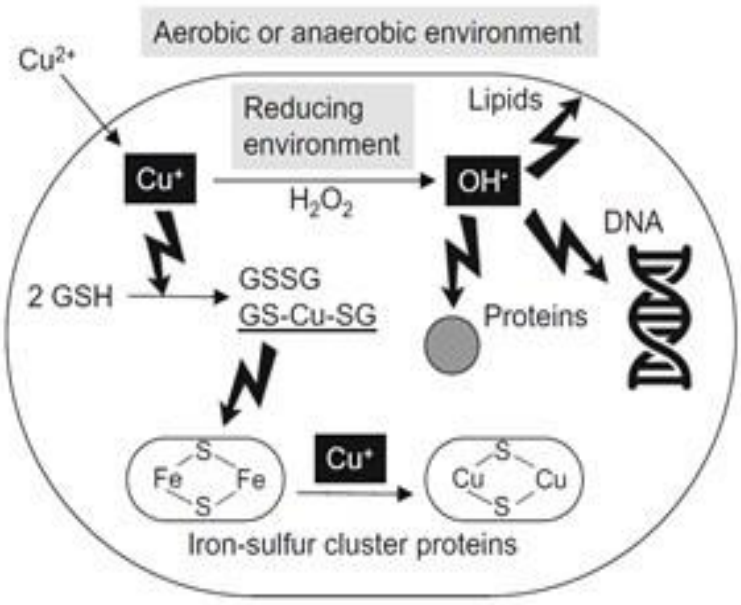


Figure 2: The mechanisms involved in the toxicity of copper nanoparticle *particles* [62].

3.10. Different methods to produce Cu-NPs coated fabrics and their effectiveness against pathogens

Two classification methods, in-situ and ex-situ, are utilized for categorizing the antibacterial functionalization of cotton and other materials using copper nanoparticles [62][63]. The process of synthesizing nanoparticles in the presence of fabric is depicted in Figure 3. This involved immersing pure cotton fabric into a copper acetate mixture, subjecting it to ultrasound waves, and subsequently introducing a reducing agent. As a result, CuO was synthesized onto the cotton fabric. X-ray diffraction was employed by researchers to confirm the presence of nanoparticles with a monoclinic crystalline structure of CuO within the fabric. The ultrasound-based functionalization represents an in-situ procedure, yielding textiles endowed with remarkable antimicrobial activity against both Staphylococcus and Gram-negative bacteria. Furthermore, this functionality exhibited efficacy even after laundering [64].

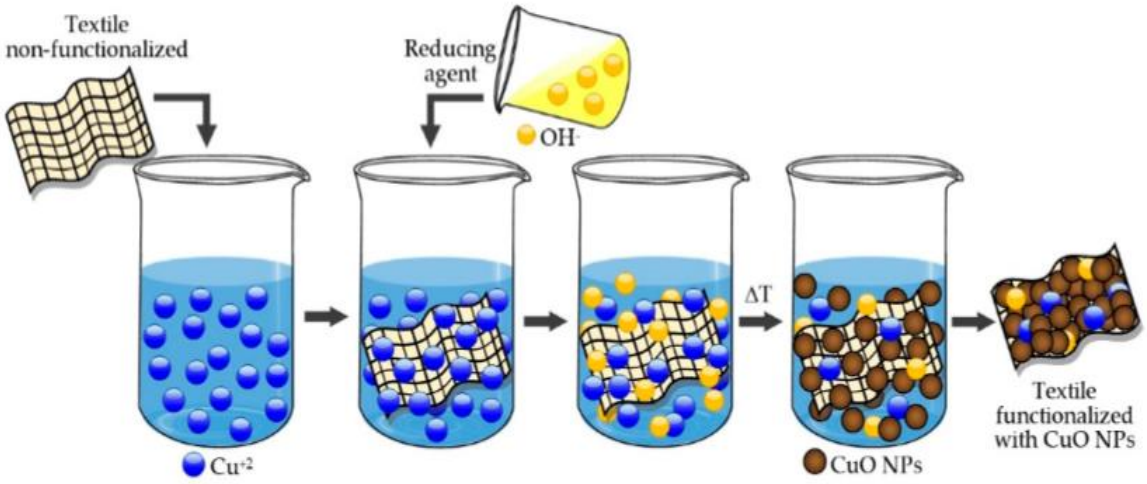


Figure 3: In situ functionalization of textile with nanoparticles [64].

Another excellent method within in-situ antibacterial functionalization is the Dip-coating technique. Researchers and investigators have employed various pre-treatments on fabrics before initiating the functionalization process. For instance, cotton cloth was laundered with a non-ionic cleanser and subsequently treated with oxalic acid concentrations to modify its surface and introduce the –COOH group, facilitating binding with Cu^{+2} ions [65]. Treating cotton fabric with ammonium hydroxide and hydrogen peroxide generates hydroxyl groups on the fabric, which interact with other chemicals involved in CuO functionalization. In a different study, woolen fabric was subjected to a non-ionic solution to reduce impurities prior to subsequent processing steps [66]. Fabric samples were immersed in copper precursor salts, which included copper ions like sulphate and acetate. Basic solutions such as sodium borohydride and sodium hydroxide from industrial sources were used to process the fabric. Rezaie's laboratory produced CuO nanoparticles within the fabric using supernatant from leaves after combustion or ash from stems (Seidlitzia Rosmarinus). Additionally, Markovic et al. reported a significant modification of cotton fabric involving the introduction of –COOH groups through the use of oxalic acid. This enhancement facilitates the absorption of Cu ions in the in-situ antibacterial functionalization process using copper oxide nanoparticles. Cotton fabric treated with higher levels of oxalic acid exhibits greater affinity for absorbing Cu ions. Scanning electron microscopy (SEM) images effectively capture the fabric's robust antibacterial activities against *Staphylococcus* and *E. coli* species [67].

Another technique, as reported by a group of researchers, involves the functionalization of cotton with CuO nanoparticles using the exhaust dyeing method. In this

approach, sodium hydroxide and copper acetate were incorporated. The resulting antimicrobial activity demonstrated that the treated cotton fabrics hinder the growth of gram-negative bacteria. The underlying mechanism of this antimicrobial effect involves the interaction between the OH- group present in both cotton fabric and CuO nanoparticles. This OH- group is inherent to cellulose, and the complex interplay between these elements could be the source of CuO nanoparticle deposition. The scanning electron microscope allows for the visualization of the morphological structure. Figure 4a depicts raw fabric without nanoparticles, while Figure 4b showcases the functionalized cotton fabric with randomly dispersed nanoparticles [68].

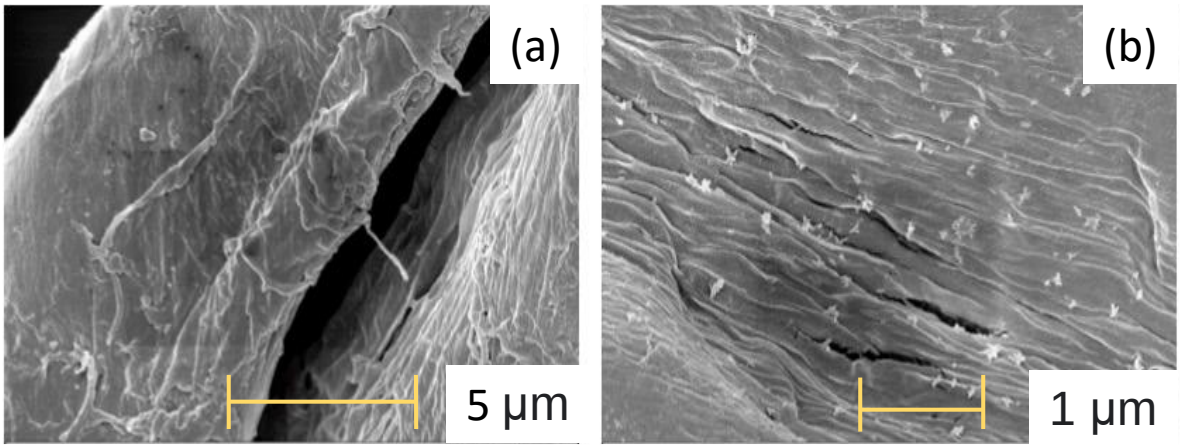


Figure 4:SEM images of untreated cotton fabric (a) and fabric with fabric coated with in-situ Cu nanoparticles growth (b) [68].

Table 2 listed the other methods besides the in-situ functionalization and describes all details about acquired functionality, size of nanoparticles, precursor, nature of fabric, and antimicrobial activity against gram-positive and gram-negative bacteria.

Table 2: The table is showing different methods to fabricate the copper nanoparticles coated antibacterial textiles.

Precursor	**Fabric type**	**size (nm)**	**Application method**	**Functionality**	**Antimicrobial activity**	**Ref.**
0.01 M Cu $(Ac)_2$	Cotton	~30	Sonochemical 20 kHz	Antibacterial activity	*S. aureus, Acinetobacter baumannii, E. coli*	[69]
0.01 M Cu $(Ac)_2 \cdot XH_2O$	PC 35:65	~80	Sonochemical installation	Antibacterial activity	*E. coli, S. aureus*	[70]
8g Cu $(CH_3COO)_2 \cdot H_2O$	Cotton	~60-80	Sonochemical	Antibacterial activity	*E. coli, S. aureus*	[71]
0.1–15%	Cotton	~40-70	Dip-coating	Antibacterial	*E. coli, S. aureus*	[72]

o.w.f $CuSO_4 \cdot 5H_2O$				activity		
2 Mmol $Cu(Ac)_2 \cdot 2H_2O$	Cotton	~40-90	Ultrasonic-mediated dip coating	Antibacterial activity	*E. coli, S. aureus*	[73]
1 mm $CuCl_2$	Cotton	~25-30	microwave irradiation (2455 MHz) + dip coating	Antibacterial activity	*E. coli, S. aureus*	[74]

The ex-situ functionalization technique has two steps, the first is the synthesis of nanoparticles and application on the textile. Following Figure 5 is representative of the ex-situ functionalization technique [75].

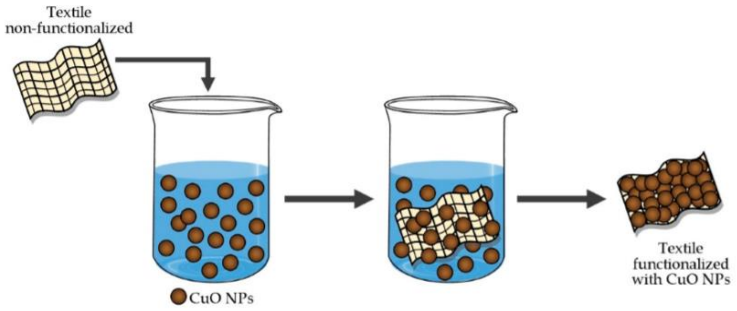


Figure 5: Representative of the second step of ex-situ functionalization of fabric with nanoparticles [75].

Thampi et al. have published a process involving chemical precipitation to prepare CuO nanoparticles, followed by their impregnation onto textile materials, whether woven or non-woven [76]. The mixture of polyethylene glycol and copper nitrate was utilized for CuO nanoparticle synthesis. The subsequent step involves immersing the textile into this solution, followed by agitation and drying. To immobilize the CuO particles, a polymeric matrix of polyaniline was created. This approach facilitates the leaching of copper ions into the environment from the functionalized fabric. CuO was introduced into the dissolved aniline and then applied to the fabric. This approach exhibits highly effective antibacterial results against both gram-positive and gram-negative bacteria. This report serves as an excellent example of a two-step ex-situ functionalization method.

Vasantharaj et al. also reported an alternative form of functionalization using the ex-situ technique. Copper sulfate solution was employed to synthesize CuO nanoparticles. The extract of *Ruellia tuberosa*, a plant with copper-toxicity-reducing properties, was utilized in this process. The synthesized CuO nanoparticles were then applied to textiles. Researchers

confirmed the composition of the fabricated nanoparticles as CuO using FTIR (Fourier transform infrared spectroscopy) and ultraviolet-visible spectroscopic techniques. The treated cotton fabric, coated with these synthesized CuO nanoparticles, demonstrated remarkable antibacterial effects against both gram-positive and gram-negative bacteria. [77].

Chemical electroless plating is an additional technique, as reported by Ali et al., employed for nanoparticle application onto textile surfaces [78]. In this approach, copper and silver nanoparticles were deposited onto the textile surface to activate it. Electroless plating was used to create a thin copper coating on the fabric. The treated fabric's antimicrobial activity was assessed and exhibited remarkable effects. Through the integration of silver and copper nanoparticles, the researchers advanced their work to produce robust multifunctional textiles. These textiles demonstrated high efficacy against pathogens, thus offering a promising avenue for antimicrobial applications [79].

Research into green synthesis has become a crucial endeavor for producing metallic nanoparticles to functionalize fabric surfaces with antibacterial properties. Utilizing plant sources for metallic nanoparticle formation offers a highly viable alternative to conventional chemical methods [80]. Green synthesis ensures reduced harmful effects for consumers, as processes like capping, reducing, and stabilizing are achieved using natural compounds. The literature extensively showcases nanoparticles prepared through green synthesis. [81].

Turakhia et al. demonstrated the synthesis of CuO nanoparticles utilizing Carica papaya leaf extract as a precursor. A solution of 0.01 M $CuSO_4$ was used. Scanning electron microscopy was employed to analyze the morphological features of the CuO nanoparticles. Figure 6a illustrates nanoparticles with a size of less than 100 nm, confirming their antimicrobial activity. Additionally, Figure 6b showcases the uniform distribution of CuO nanoparticles on the textile surface [82].

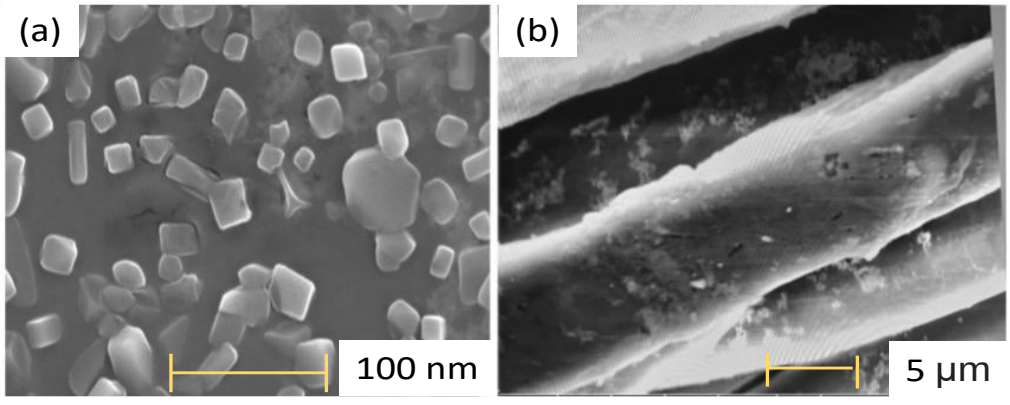


Figure 6: Representative of the second step of ex-situ functionalization of fabric with nanoparticles [82].

The agar diffusion method was employed to assess the antibacterial efficacy of CuO nanoparticle-treated fabric against both gram-positive and gram-negative bacteria. Bacillus species, *E. coli*, and Salmonella species were chosen as the test organisms. Notably, Figure 7 vividly displays the robust antimicrobial activities exhibited by the treated fabric [82].

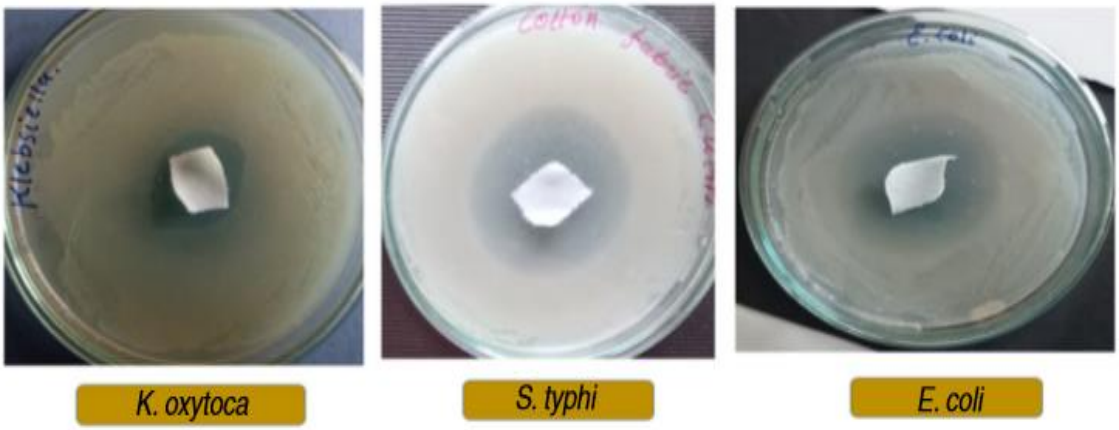


Figure 7: The antimicrobial activity of CuO-treated fabric against *Klebsiella oxytoca, salmonella typhi*, and *Escherichia coli* [82].

The green synthesis of CuO nanoparticles by the use of *Tinospora cadifolia* was published by Sharma et al [83]. The capping agent employed was derived from the leaf extract of the mentioned plants, while copper chloride solution served as the precursor. A dipping method was employed to imbue the synthesized nanoparticles onto cotton fabric. Scanning electron microscopy was utilized to examine both treated fabrics, with and without nanoparticles, revealing the spherical morphology of the nanoparticles (Figure 8 A-D). The nanoparticle-treated fabric exhibited remarkable antimicrobial effectiveness against both gram-positive and gram-negative bacteria. This efficacy was quantified through the measurement of inhibition zones, as depicted in Figure 9 [83].

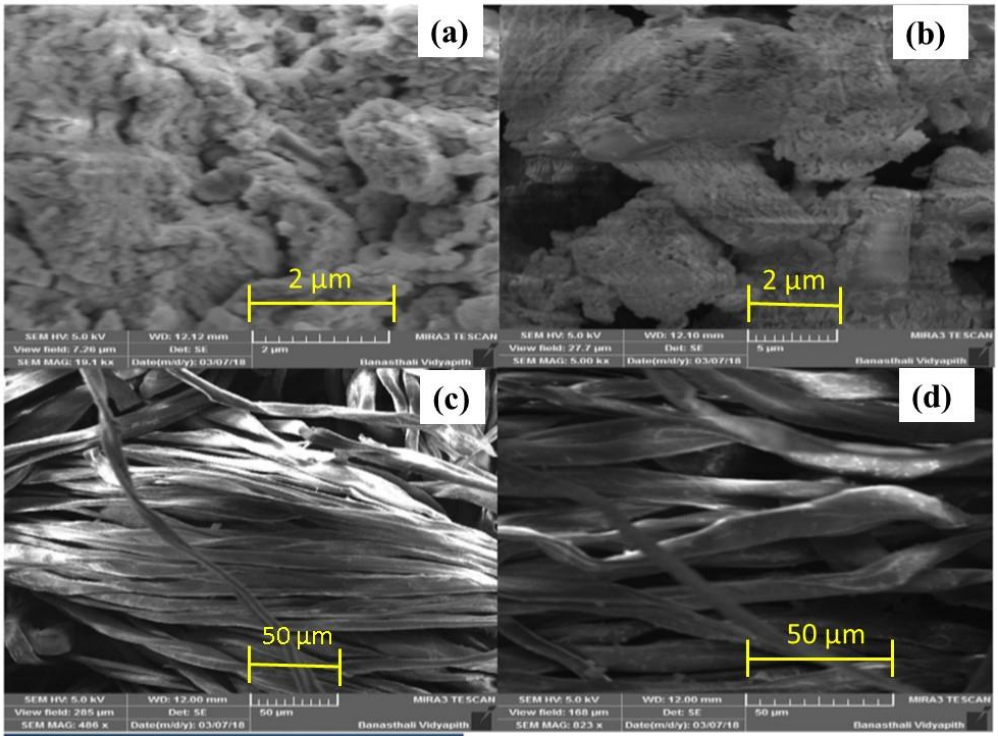


Figure 8: SEM images of Cu nanoparticles A and B, untreated cotton fabric (C), and fabric treated with Cu-NPs (D) [83].

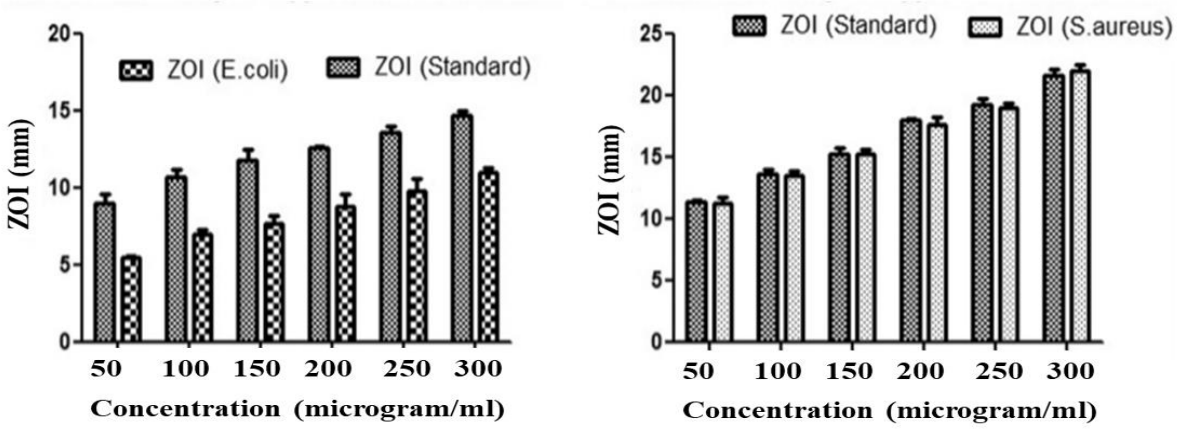


Figure 9: Zone of inhibitions (ZOI) shown by fabric treated with Cu-NPs against *E. coli* (left) and *S. aureus* (right) [83].

Cinnamon bark extract emerged as a promising non-toxic reducing and stabilizing agent employed by a team of researchers for capping and fabricating Cu nanoparticles. Utilizing copper nitrate solution as the precursor, the process was further aided by citric acid to enhance the reducing effect of cinnamon during synthesis. This combined approach resulted in improved surface morphology of the nanoparticles. The scanning electron microscope image in Figure 10 showcases the surface characteristics of the produced copper

nanoparticles, exhibiting a consistent and spherical structure, facilitated by the presence of citric acid [84].

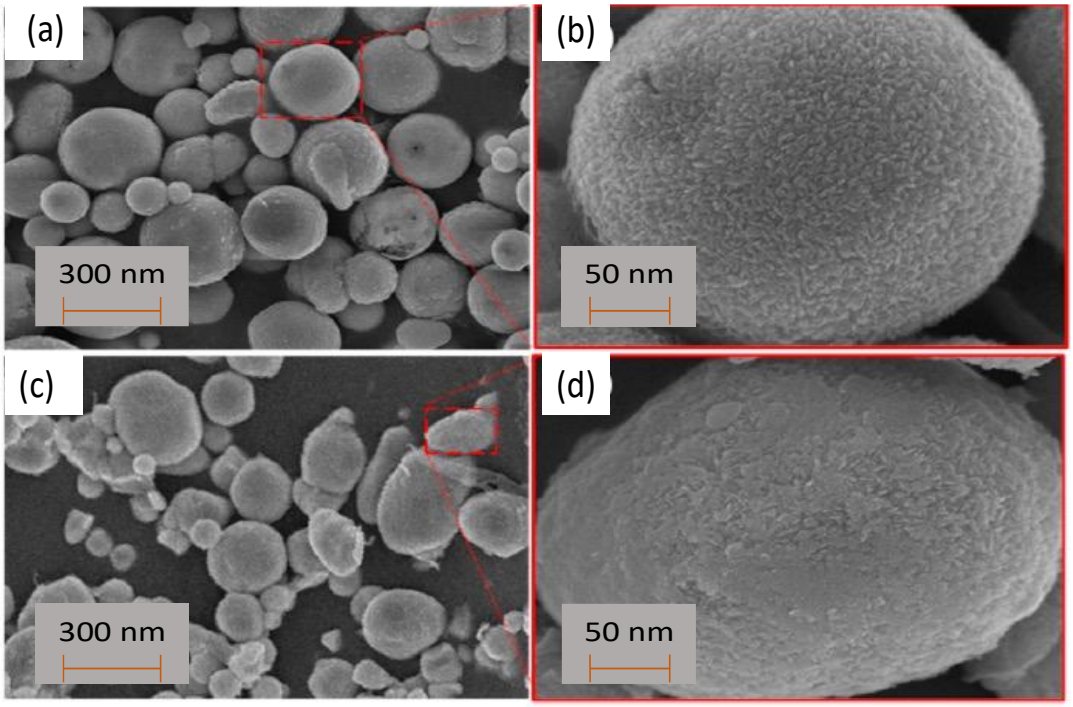


Figure 10: SEM images of citric acid-mediated Cu NPs (a-b) and in the absence of citric acid (c-d) [84].

The pad dry cure procedure is used to form the cotton fabric. The microbial inhibition zone was used to check the antimicrobial activity of treated fabrics. The samples have shown very good inhibition zones in Figure 11 [82].

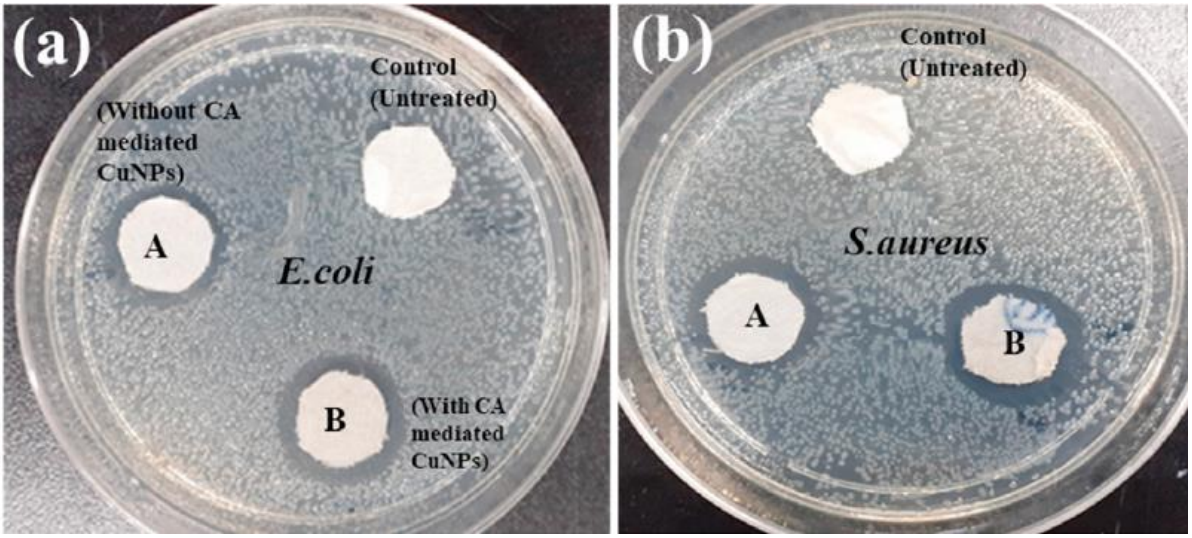


Figure 11: Antibacterial activity (ZOI) of Cu NPs treated fabric against *E. coli* (a) and *S. aureus* (b) [82].

In a separate investigation, Vasantharaj et al. [85] explored the utilization of Ruellia tuberosa leaf extract for the synthesis of copper nanoparticles. A copper sulphate solution served as the precursor for this process. Transmission electron microscopy revealed the presence of rod-shaped copper oxide nanoparticles ranging in size from 20 to 100 nm. The nanorods' formation was attributed to the phytochemical particles present in Ruellia tuberosa (Figure 12 a-b). Scanning electron microscopy was employed to analyze cotton fabrics, both treated and untreated with copper oxide nanoparticles. The presence of CuO nanoparticles on the surface of the thin cotton fibers was evident in the scanning electron microscope images (Figure 13 a), while untreated cotton fabrics were used as the control (Figure 13b). Furthermore, the antimicrobial activity of these treated nanoparticle fibers was tested against gram-positive and gram-negative bacterial species (Figure 14) [85].

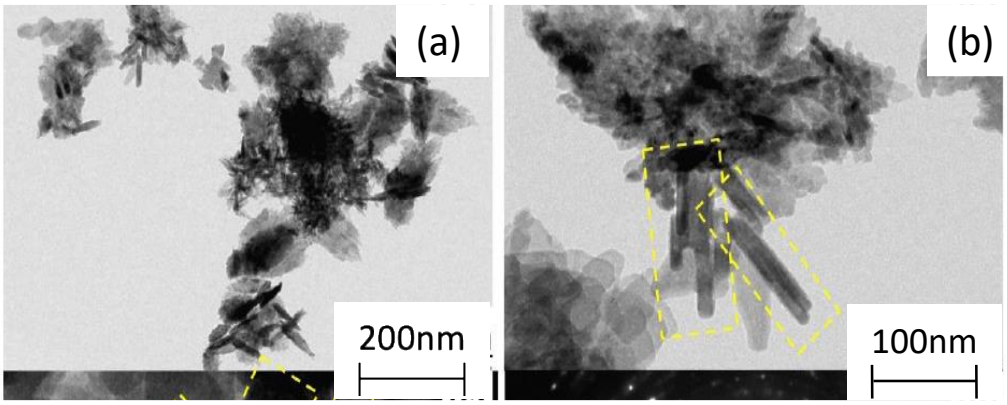


Figure 12:TEM images of rod-shaped biologically synthesized copper nanoparticles [85].

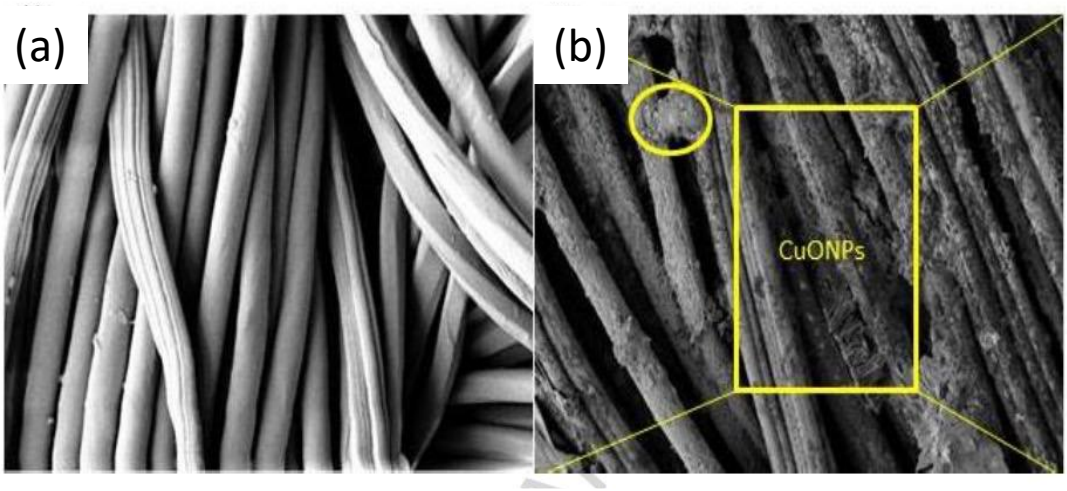


Figure 13:SEM images of untreated cotton fabric (a) and fabric treated with CuO nanoparticles (b) [85].

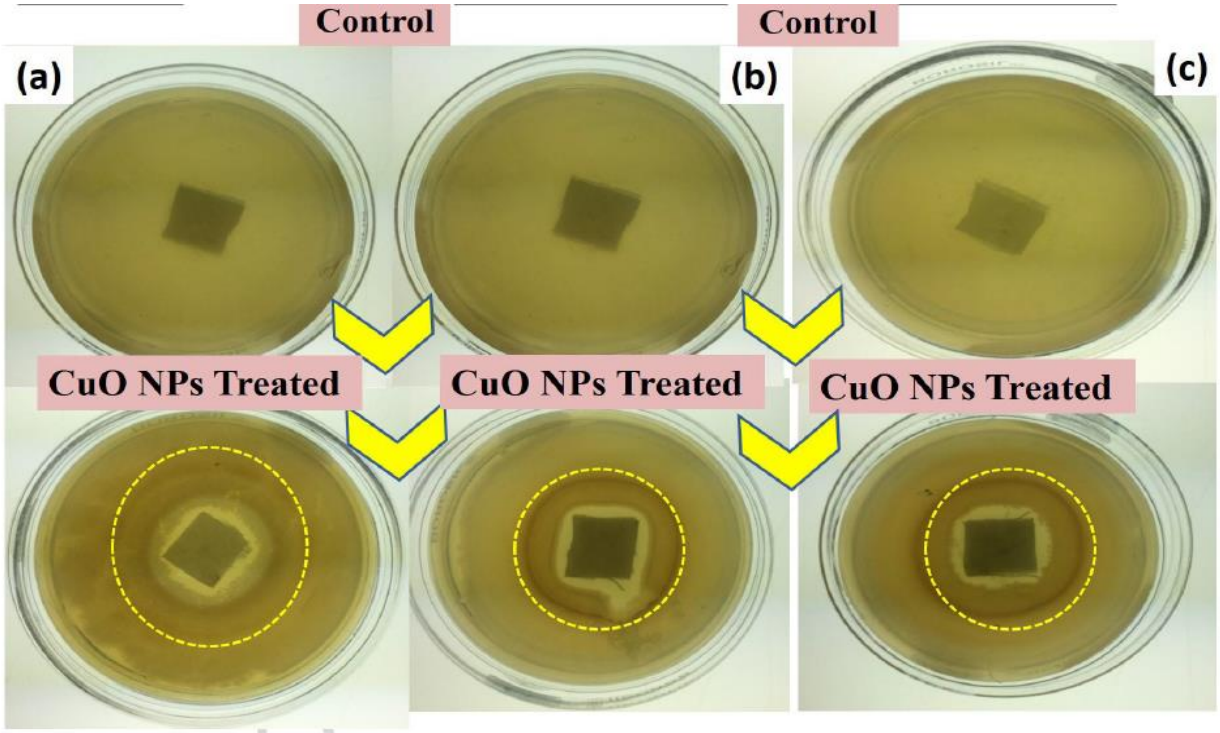


Figure 14: The ZOI of untreated and CuO NPs treated cotton fabrics against *E. coli* (a), K. pneumoniae (b), and *S. aureus* (c) [85].

. Vinothkhanna et al. employed R.cordifolia bark extract and copper sulphate solution as the precursor for the synthesis of copper oxide nanoparticles. The biological synthesis of these nanoparticles was carried out using this approach. The antibacterial potential of these particles was investigated, with energy dispersive X-ray (EDX) analysis confirming the presence of copper oxide nanoparticles. Morphological assessment was conducted using scanning electron microscopy (SEM). The CuO nanoparticles treated with R.cordifolia extract exhibited irregular and rough shapes, with some showing spherical morphologies (Figure 15). The average particle size was determined to be 50.72 nm (Figure 16). This particle size aligns closely with those produced from *Sida acuta* extract, which also measures around 50 nm [86].

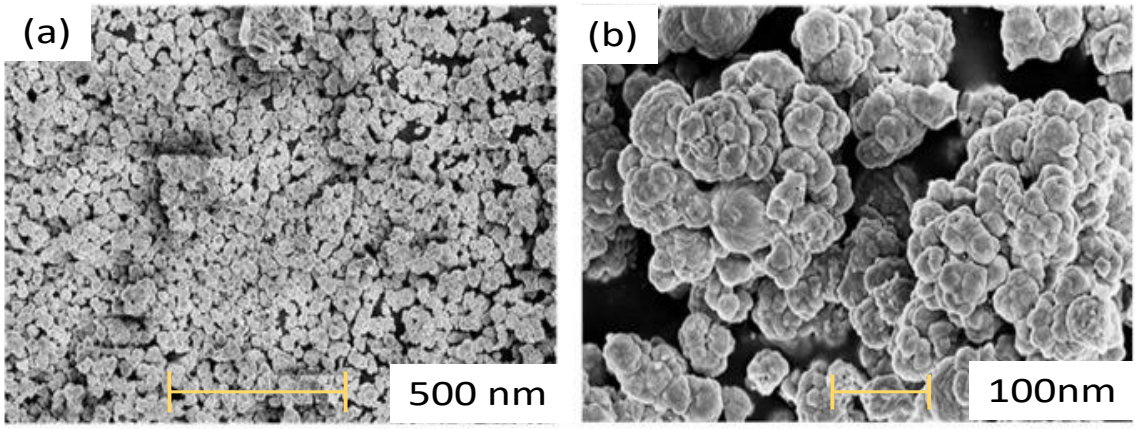


Figure 15:SEM images of CuO nanoparticles synthesized from *R. cordifolia* plant extract [86].

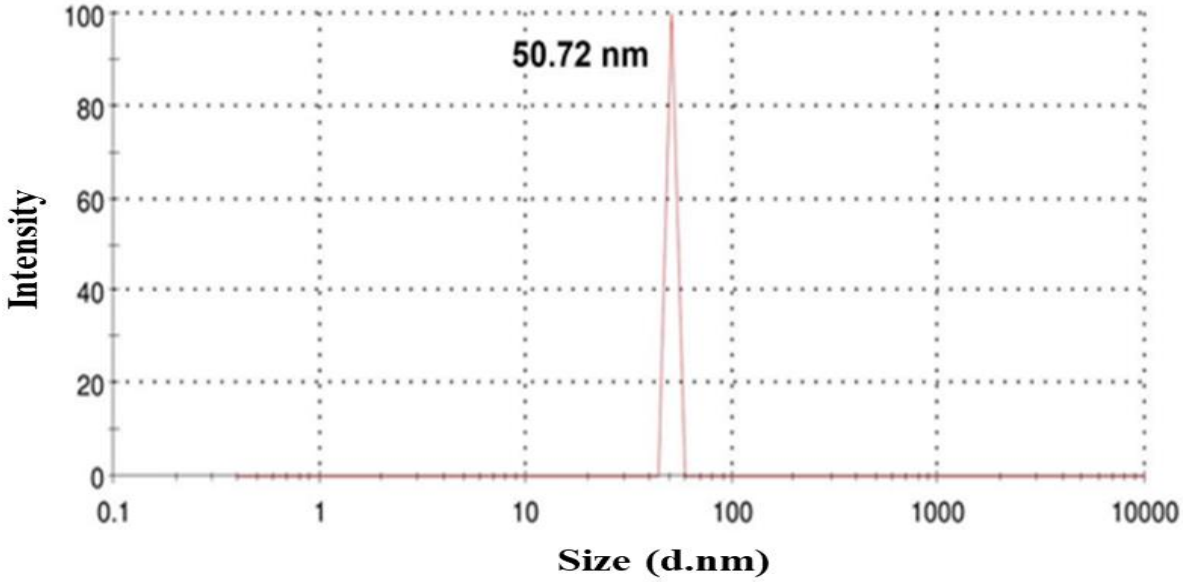


Figure 16: Average particle size for the CuO nanoparticles synthesized from *R. cordifolia* plant extract [86].

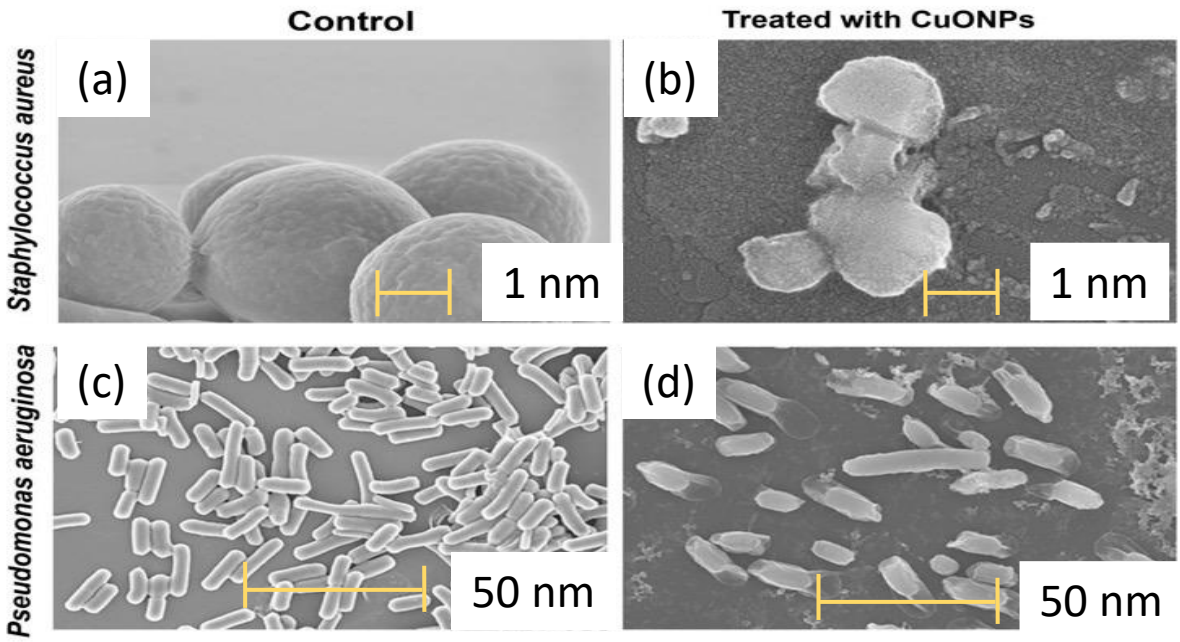


Figure 17:SEM images showing live and dead bacterial cells for both untreated and treated cotton fabrics [86]. *1 nm, 1 nm, 50 nm, 50 nm*

The antibacterial activity was examined against gram-positive and gram-negative bacteria. Both groups of bacteria were significantly inhibited by the CuO nanoparticles. The scanning electron microscope images in Figure 17 are representative of cotton fabrics.

3.11. Testing methods for the evaluation of antimicrobial activity

To evaluate the effectiveness of antimicrobial textiles in terms of their antibacterial, antiviral, and antifungal activities, various testing protocols have been developed. These protocols can be broadly categorized into qualitative and quantitative testing methods. Here, we provide a brief overview of the methods used for each category.

3.11.1. Qualitative testing protocol

Qualitative testing methods include the agar diffusion testing methods such as AATCC 147-2004 (American Association of Textile Chemists and Colorists), SN 195920-1992 (Swiss Norm), and JIS L1902-2002 (Japanese Industrial Standards). These methods offer a qualitative assessment of antimicrobial activity and are particularly suitable for rapidly screening a large number of textile samples. In these tests, microbial cells are inoculated onto nutrient agar plates, which are then placed in direct contact with the textile specimens. After incubation at 37°C for 18–24 hrs, the plates are checked for microbial growth directly beneath the textiles and around the fabric samples (zone of inhibition). Antimicrobial activity is indicated by the absence of microbial growth directly beneath the fabric swatch. Whereas if

the antimicrobial agent is strongly bonded to the textiles (covalent linkage), it will not diffuse into the agar, resulting in the lack of zone of inhibition. If the antimicrobial agent will diffuse in the agar media, a zone of inhibition (ZOI) appears, and its diameter gives some measure of the potential of antimicrobial activity. For the determination of the diameter of the zone of inhibition, equations are employed. The methodology of all the above-mentioned methods is the same however calculation method varies for all methods. The AATCC-147 method is preferred as this method provides comparatively reliable results in terms of the diameter of the zone of inhibitions.

3.11.2. Quantitative testing protocols

- ***Antibacterial and antifungal assays***

Quantitative test methods for assessing the antibacterial and antifungal properties of finished textiles include AATCC 100–2004, SN 195924–1992, JIS L1902-2002, and ISO 20743 Transfer method. These techniques provide quantitative measurements of antimicrobial efficacy but typically require more time compared to agar diffusion testing. In these methods, a small amount of microbial inoculum (e.g., 1 mL) is evenly distributed on test fabric specimens. This step ensures direct contact between the microbes and the fabric samples. The fabric with the microbial suspension is then incubated for specified time periods according to the relevant standard. The total number of inoculated microbes and the surviving colonies are determined using serial dilution and plating on agar media plates. The antibacterial activity is assessed by comparing the counts of inoculated and surviving microbial colonies after specific incubation periods. The results are expressed as a percentage reduction or log reduction. To ensure that the reduction in microbial count is attributed to the antimicrobial finishing, appropriate control samples that have undergone the same processing steps except for the antimicrobial treatment must be included in each experiment. The choice of an appropriate calculation equation is also crucial for accurate interpretation.

- ***Antiviral assays***

The standard protocol ISO 18184:2019 have been extensively employed for the quantitative evaluation of antiviral textile. In this method, viral cell cultures are seeded at specific concentrations in 96-well plates at cells in a suitable growth media (usually DMEM) and incubated at standard conditions of temperature, humidity, and CO_2. Each treated fabric was cut into sizes and placed on the top of the 1-cm diameter vials. Then, the viral stocks (0.1 mL) are passed through the fabrics, and collected virus stocks in vials are sterilized by passing from a filter of specific pore size. The virus is then serially diluted usually from 10^{-1}

to 10^{-9} in 10-fold increments. Each dilution is inoculated in viral cell cultures and incubated at standard conditions. Viral titers in the cell culture are calculated by Behrens and Karber's method.

3.11.3 Determination of minimum inhibitory concentration (MIC)

For determining the Minimum Inhibitory Concentration (MIC) of antimicrobial agents, two common methods are the broth tube dilution assay and the Liofilchem strip test. The MIC value signifies the lowest concentration at which the growth of the tested microorganisms is inhibited by the antimicrobial agent. In the broth tube dilution assay, cultures of the microorganisms are exposed to varying concentrations of the antimicrobial agent in fresh growth media. The concentration at which no growth is observed is considered the MIC value for that specific antimicrobial agent. On the other hand, the Liofilchem strip test is less economically viable due to the high cost of the strips used in the test. As a result, the broth dilution assay is often preferred. This method doesn't require any specialized materials like the strip test, making it a more practical and cost-effective approach for determining MIC values.

3.12. Applications of bioactive textiles

Indeed, antimicrobial textiles serve a wide range of applications, encompassing clinical settings, first aid scenarios, and hygiene practices. However, their utility extends even further into the realm of bio-functional textiles. These innovative materials involve the integration of advanced pharmaceutical nanocarriers with conventional fabrics, resulting in wearable platforms for drug delivery. This convergence of textiles and drug delivery offers intriguing prospects, potentially enhancing the penetration of therapeutic agents through the skin while concurrently mitigating the risks associated with toxicity. [87]. Antimicrobial textile materials have been utilized as a matrix for various active chemicals, allowing them to be released gradually. These textiles can be utilized as a carrier for melanoma, aromatherapy, atopic dermatitis, psoriasis treatment, opioids, and hormone therapy among other aspects [88]. Recent research [89] demonstrated that silver-impregnated fabrics could help those with atopic dermatitis. The ability of Ag nanoparticles to adhere to cotton fabric and penetrate it suggested that they could be employed as a wound treatment. These highly effective antibacterial cotton fabrics have no toxicity when tested on human cells [90]. Cotton wound dressings were designed to cure fungal infections on the skin. This wound dressing contains ketoconazole and cyclodextrin, which showed a controlled and delayed release of these antifungal chemicals to kill *Candida albicans* and *Aspergillus Niger* skin fungus [91].

Antimicrobial textiles hold considerable significance across various domains beyond everyday wear. A recent evaluation has been conducted concerning the applicability of antimicrobial textiles in the context of extended space travel. While the space travel environment is generally pathogen-free, the extended use of spacesuits without access to laundry facilities poses challenges. With limited clothing on hand during missions, the development of antimicrobial textiles gains prominence in addressing microbial growth within spacesuits during long-duration space flights. [91]. Microbes may grow when they come into contact with human skin, and there are no choices for cleaning clothes effectively. Antimicrobial textiles could reduce or remove the need for cleaning in space [92].

The outbreak of COVID-19 has spurred a significant surge in the production and utilization of bioactive textiles, including items like surgical masks, gloves, protective clothing, and footwear. Antimicrobial sportswear has gained traction due to its potential in curbing pathogen growth and minimizing sweat-related odors. Sensory evaluations of odor-control textiles revealed their reduced intensity compared to untreated counterparts. Innovative solutions, such as flexible antimicrobial sleeves, have been developed to prevent pathogen transmission through sneezing or coughing. Additionally, the demand for breathable and antimicrobial undergarments, including bras and men's undergarments, has risen sharply in response to health and hygiene concerns.

The concrete applications of bioactive textiles in different sectors are given below,

- **Commercial:** carpets, automobile seat coverings, military textiles, tents, uniforms.
- **Health care:** wound dressings, earbuds, compression bandages, masks, surgical gowns, lab coats, scrub suits, protective kits, and overalls.
- **Apparel:** jackets, sanitary pads, compression garments, caps, activewear, undergarments, winter-wears
- **Households:** bedding, carpet, table and chair coverings, curtains, mop, pillows, towels

However, the limitation of currently available bioactive textiles is their poor washing durability. The use of such bioactive textiles is unsustainable due to the release of antimicrobial agents from the fabric into the environment. The side effects of these antimicrobials were initially ignored, but their harmful effects on the environment and human health have now drawn increasing attention. The idea of safe bioactive agents and textiles emerged after the 1962 publication of Rachel Carson's book Silent Spring. Different approaches have been developed by researchers such as the use of crosslinkers/binders,

modification of textiles, and antibacterial agents to introduce reactive sites on their surfaces. The washing durability has been improved sufficiently by using these techniques. However, robust immobilization of antibacterial agents on textiles still presents a great challenge to researchers [93].

4. EXPERIMENTAL PART

In this study, a plain-woven cotton fabric with an areal density of 220 g/m^2 was employed as the substrate for producing antibacterial fabrics. The chemicals used for the synthesis and deposition of cuprous oxide (Cu_2O) had 99.99% purity. Table 3 lists the components that were used in this study. Reactive blue 4 (35% dye content) was purchased from Sigma Aldrich. Triclosan (97%) was procured from TCI Japan.

Table 3: List of materials used in present study.

Materials Description	Source
Plain woven 100 % cotton bleached fabric, areal density 220 g/m^2	Licolor, a.s. Czech Republic
Sodium Potassium tartrate	ACS reagent
Copper sulfate pentahydrate	ACS reagent
Ascorbic acid	ACS reagent
Glucose	Aldrich Reagent-Plus
$Na_2S_2O_4$ (Sodium dithionite)	ACS reagent
Reactive Blue 4	Sigma Aldrich
Triclosan	TCI Japan

The plain-woven standard bleached cotton fabrics with weave structure (EPI × PPI = 28 × 23, warp and weft count = 23s Ne, GSM = 150 g m^{-2}) having a thickness of 0.35 mm was used. The calculated volume porosity is 72%. The woven fabric was purchased from Licolor, a, s. Figure 18 is showing the macroscopic image of plain-woven cotton fabric taken by ProgRes CT3 macroscope.

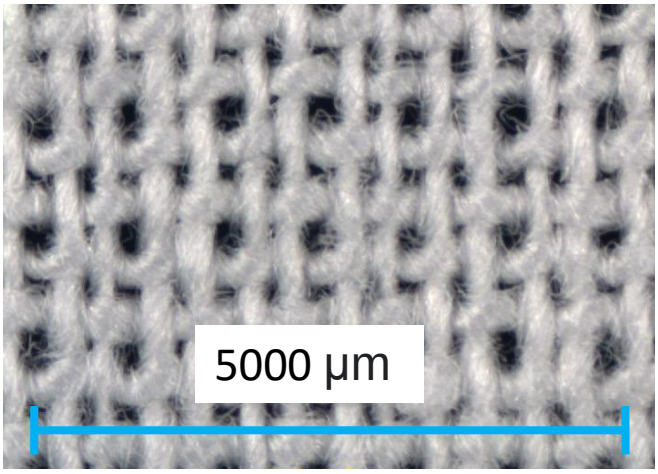


Figure 18: Microscopic image of plain-woven cotton fabric.

The experimental part is composed of three parts.

- The first part, the Formation of Cu_2O particles was done by the Fehling solution method (by using three different reducing agents)
- The second part, the deposition of cuprous oxide particles was done on woven cotton fabric and studied their antipathogenic (antibacterial, antifungal, and antiviral) properties.
- The third step was to dye the already cuprous oxide-coated fabrics with an antibacterial dye

4.1 Preparation of cuprous oxides particles

Cuprous oxide particles Cu_2O were prepared by combinations of two Fehling (A & B) solution by using three different reducing agents (Glucose, Ascorbic acid, and Sodium hydrosulphite). Fehling solution A and Fehling solution B were prepared separately. For the preparation of Fehling A, 34.64 grams of $CuSO_4.5H_2O$ was dissolved in 500 ml of distilled water and stirred continuously. The Fehling B solution was prepared by dissolving 70 grams of NaOH and 175 grams of Sodium Potassium tartrate were dissolved in 500 ml of water. Subsequently, we took 100 ml of each Fehling A and Fehling B in a cleaned round bottom flask and heated it to 95°C with continuous stirring. Then 10 grams of reducing agent (glucose) was dissolved in 100 ml of water and added into the above solution (Fehling A and Fehling B in a cleaned round bottom flask and heated to 95°C). The color of the solution turned from blue to red and a large amount of precipitate was formed in the bottom of the flask. The precipitate was centrifuged and washed 3- 4 times with deionized water. The same procedure was also repeated with the other two reducing agents (ascorbic acid, sodium hydrosulphite). Hence, we have three types of Cu_2O particles, prepared with three different types of reducing agents (Glucose, Ascorbic acid, and Sodium hydrosulphite).

4.2 Deposition of Cu_2O particles on cotton fabric

Before the deposition of cuprous oxide nanoparticles substrate was pre-treated. Pre-treatment was done with citric acid. A solution of 20 g/L citric acid was made and fabric was dipped in it at 80°C for 2 hours, then washed and dried at 90 °C for 50 minutes. After pre-treatment citric acid is not a part of cotton fiber. Citric acid is there as an ion exchanger. During pre-treatment, the sodium and other ions were removed from fibers and replaced by H^+ ions from the citric acid solution. By this pre-treatment are the fibers activated by a

change of zeta potential. Cu particles are attracted after this preparation from solution effectively to fiber surfaces by electrical forces.

As mentioned earlier, we have prepared three different types of Cu_2O particles with three different types of reducing agents (Glucose, Ascorbic acid, and Sodium hydrosulphite). In the next step, three different concentrations (1g, 0.5g, 0.25g) of each type of Cu_2O were applied to pre-treated cotton fabric. The concentrations (1g, 0.5g, 0.25g) of Cu_2O centrifuged particles were dispersed in 200 ml of water**.** Cotton fabric was dipped in each solution for 30 minutes then pad and dry at 90°C for 20 minutes. We made 3 samples against each selected reducing agent. The same procedure was adopted for each reducing agent (Glucose, Ascorbic acid, and Sodium hydrosulphite)**.** Hence, we developed a total of 9 samples for three reducing agents as shown in Table 4 below.

Table 4: Design of experiments for the developed samples.

No of samples	Reducing agent	Code of reducing agent	Concentration of Cu_2O particles
1	Glucose	G1	1g/200ml
2	Glucose	G2	0.5g/200ml
3	Glucose	G3	0.25/200ml
4	Ascorbic acid	A1	1g/200ml
5	Ascorbic acid	A2	0.5g/200ml
6	Ascorbic acid	A3	0.25/200ml
7	Sodium hydrosulphite	S1	1g/200ml
8	Sodium hydrosulphite	S2	0.5g/200ml
9	Sodium hydrosulphite	S3	0.25/200ml

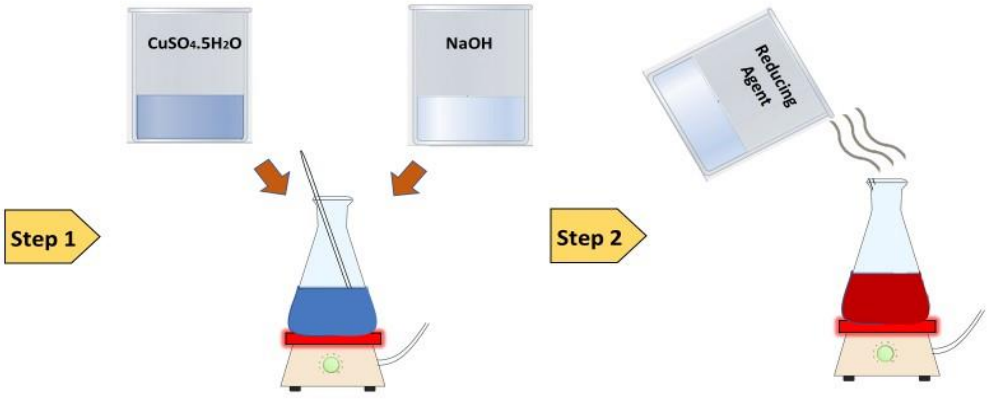


Figure 19: Formation of cuprous oxide particles and application on cotton fabric.

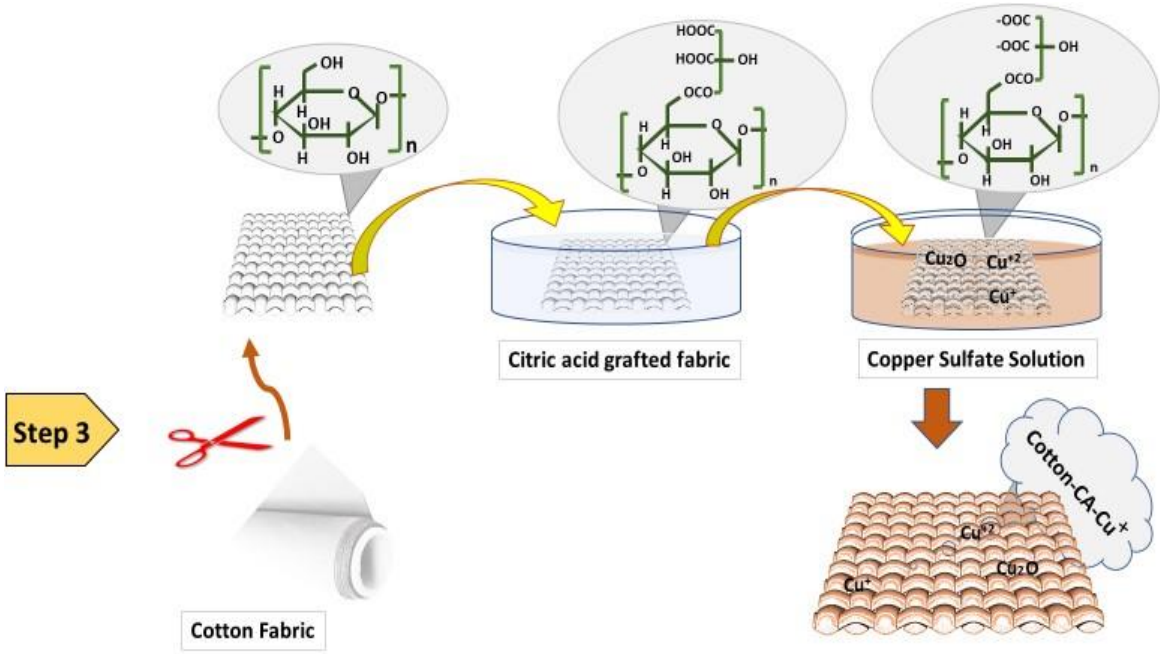


Figure 20: Schematic illustration of the three-step process for the synthesis of antibacterial cuprous oxide particles and deposition on cotton fabric.

4.3 Preparation of cuprous oxides particles and deposition on cotton for the second part

Cuprous oxide particles (Cu_2O) were synthesized by combinations of two Fehling (A & B) solutions and three separate reducing agents, namely Glucose, Ascorbic acid, and Sodium hydrosulphite. Separate preparations of Fehling solutions A and B were made. For the preparation of Fehling A, 69.28 grams of $CuSO_4.5H_2O$ was dissolved in 1 liter of distilled water and stirred continuously. Then added a few drops of H_2SO_4 (we added 5 drops). 350 g of Sodium Potassium tartrate and 140 g of NaOH were mixed in 1 liter of distilled water to prepare the Fehling B solution. Fehling A and B solutions were mixed in a 1:1 ratio. Then reducing agent (Glucose) was immediately added to the mixed solution (2.5% of the total weight of the mixed solutions - Fehling A + Fehling B).

The solution of fehlings and glucose was applied to cotton fabric using the padding method (contact time between the fabric and solution was max. 10 s) on a padder and the wet allowance is approximately 75 %. After squeezing the excess solution from the fabric, the fabric is placed in a heat press for 4 minutes at 50 °C. After removing the fabric from the press, the sample must be washed, preferably in boiling water, to remove the alkali and then dried. The same process was conducted for the other two reducing agents i.e., sodium hydrosulphite and ascorbic acid.

4.4 Functionalization of Reactive Blue 4 dye with Triclosan

Functionalization of Reactive Blue 4 **(2)** dye with triclosan **(1)** was carried out in a single-step reaction. An equimolar amount (20 mM) of both reactants was taken. In a round bottom flask, 13.6 g of reactive blue 4 dye was added and dissolved in 100 mL of distilled water (solution A). Solution A was refluxed and the temperature was maintained at 40-45°C. In a 100 mL beaker containing 20 mL of methanol, 5.79 g of triclosan (antibacterial agent) was added and stirred (solution B). The solution B was slowly added to solution A. The pH of the solution was kept neutral using 3% w/v solution of sodium carbonate. The progress of the reaction was monitored through Thin Layer Chromatography (TLC) using ethyl acetate and petroleum ether (70:30) solvent system. The reaction mass was stirred at 45°C until the disappearance of triclosan spot on TLC plate which confirmed the successful completion of the reaction and product formation. Functionalized reactive dye **(3)** was filtered and dried in the oven at 40°C. The proposed condensation reaction showing the formation of functionalized dye is given in Figure 21.

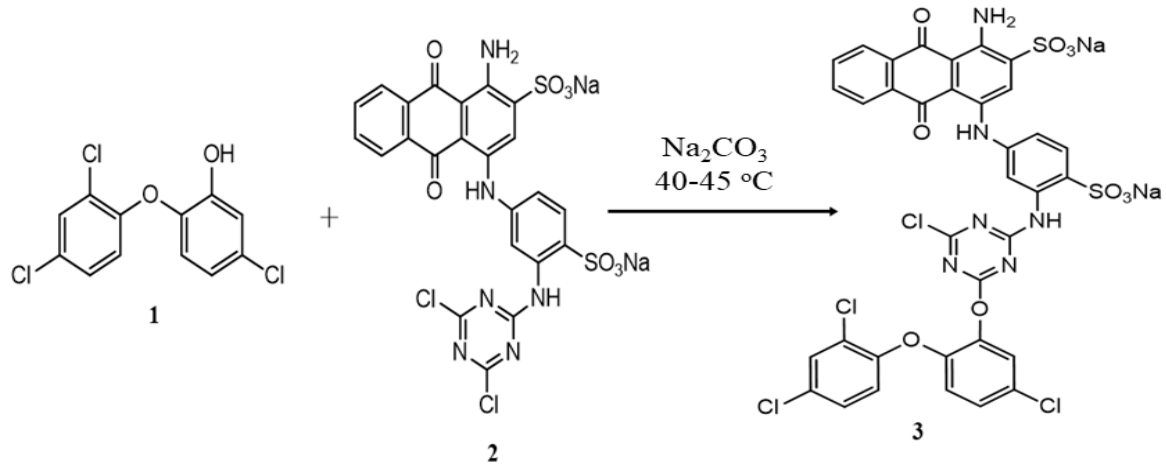


Figure 21: Antibacterial functionalization of Reactive Blue 4 dye with Triclosan.

4.5 Application of functionalized dye on Fabric

The functionalized reactive dye was applied to copper-coated cotton fabric through the exhaust dyeing method. For this purpose, an H-T dyeing machine was used. 3% dye shade (o.w.f) was applied on the fabric with a material-to-liquor (M: L) ratio of 1:50. Dyeing of the fabric was started at room temperature which was gradually increased to 70-80 °C. Electrolytes (sodium sulfate, 40 g/L) and alkali (1 g/L sodium hydroxide and 15 g/L sodium carbonate) were added portion-wise in the dyebath at different time intervals for proper exhaustion and fixation of dye. The dyeing of the fabric was continued for 60 minutes. After that, the dyed fabric was removed from the dyebath and rinsed with tap water followed by hot

was at 90 ^{o}C for 10 minutes to wash away the unfixed dye molecules from the fabric. The possible formation of a covalent bond between the chlorine atom of the triazine reactive system of dye and hydroxyl groups of cotton fabric. The schematics showing the application of modified dye on copper-treated fabrics are shown in Figure 22.

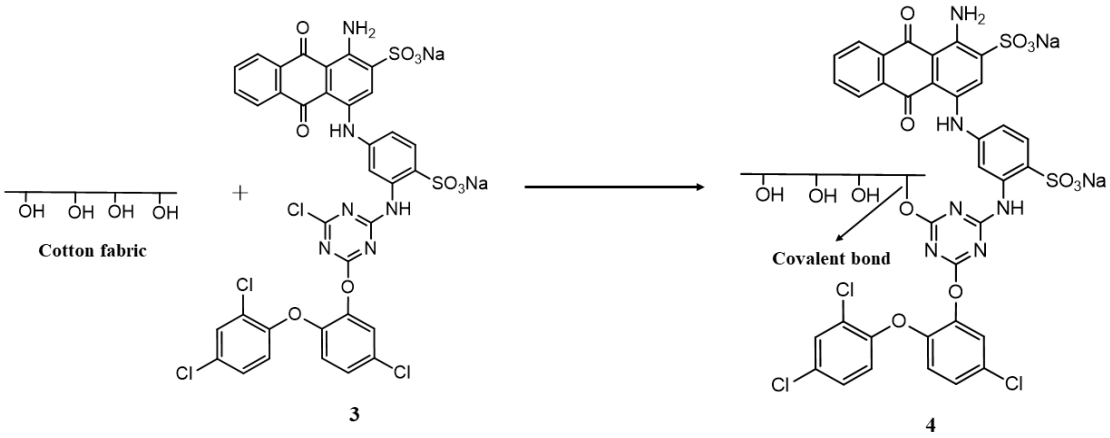


Figure 22: Schematic showing covalent bond formation between functionalized reactive dye and cotton fabric.

Hence, we developed a total of 6 samples for three reducing agents (3 are dyed and 3 are undyed). The plan of the experiments is given in Table 5 below. Figure 23 Schematic illustration of the functionalization of dye and Figure 24 is a Schematic illustration of the subsequent dyeing on Cu_2O coated fabrics. Note: Design of experiments is statistical methodology deal with mathematical modeling.

Table 5: Plan of experiments for the developed samples.

No of samples	Reducing agent	Applicant of Dye	Sample code
1	Glucose	No	G
2	Glucose	Yes	DG
3	Ascorbic acid	No	A
4	Ascorbic acid	Yes	DA
5	Sodium hydrosulphite	No	S
6	Sodium hydrosulphite	Yes	DS

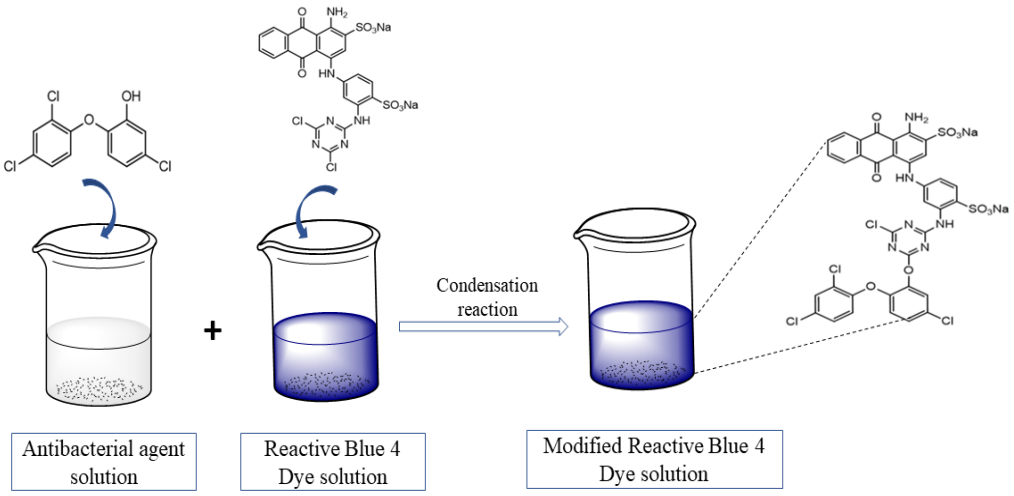


Figure 23: Schematic illustration of the functionalization of dye.

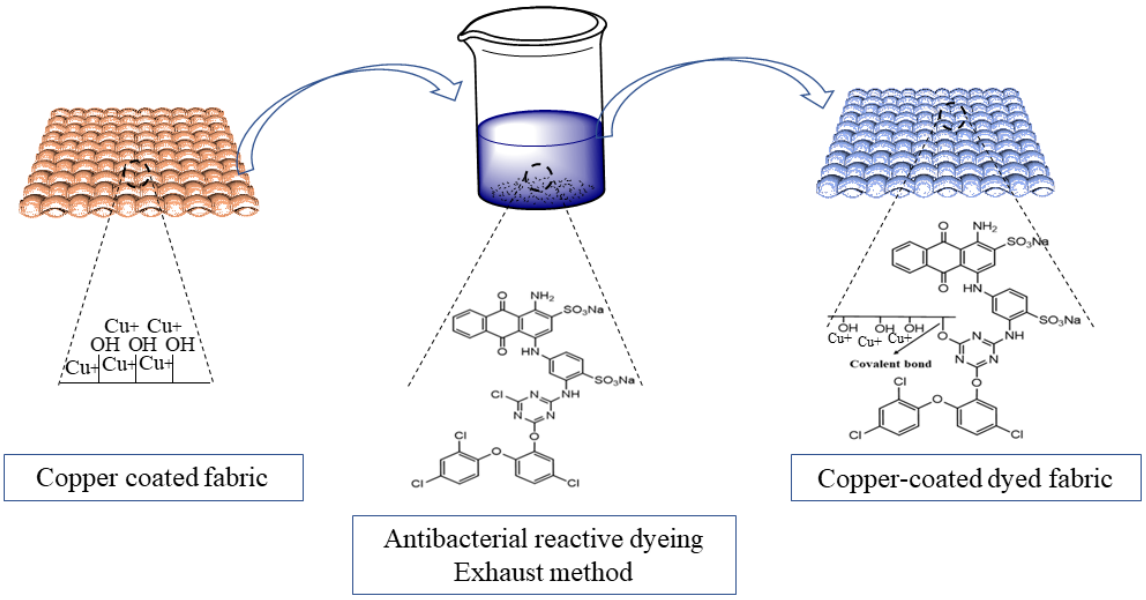


Figure 24: Schematic illustration of the subsequent dyeing on Cu_2O coated fabrics.

4.6 Surface characterizations

The morphological characteristics of cuprous oxide nanoparticles coated on the surface of cotton fabric were examined through scanning electron microscope (SEM) from FEI Quanta 50, while XRD analysis was performed with a diffractometer equipped with a conventional X-ray tube Cu Ka1 radiation (1.54 A˚) power condition (40 kV/30 mA). The

XRD pattern was measured in the 2θ range 10–80° with a step size of 0.02°. The dynamic light scattering (DLS) theory from Malvern Zetasizer, from Pan Analytical X'pert PRO tools. ^{13}C-NMRspectra were recorded using Bruker Advance Spectrophotometer which operates at 600 MHz and tetramethylsilane as an internal reference. The identification of functional group-modified reactive dye was done using FTIR spectra recorded on the FTIR Perkin Elmer spectrophotometer.

4.7. Dye exhaustion, fixation, and total fixation measurement

Exhaustion, fixation, as well as total fixation amount for the modified dye were determined using the method described in [94], following equations were employed for calculations of exhaustion

$$\%E = [(C_1 - C_2)/C_1] \times 100 \quad (2)$$

Here, C_1 [94] and C_2 [94] represent the concentration of dye in solution before and after dyeing. Let C_3 [94] shows the concentration of extracted hydrolyzed dye from cotton fibres to the bath. Portion of covalently fixed dye is then can be calculated as

$$\%F = [(C_1 - C_2 - C_3)/(C_1 - C_2)] \times 100 \quad (3)$$

Portion of hydrolyzed dye on fabric is calculated as

$$\%H = [C_3/(C_1 - C_2)] \times 100 \quad (4)$$

4.8 Fastness properties

The light, rubbing, and washing fastness properties of the copper-treated dyed fabric samples were assessed. ISO 105-X12, ISO 105-C06, and ISO 105-B02 standards were followed for the evaluation of rubbing fastness and washing fastness, and lightfastness, respectively.

4.9 Colorimetric data measurement

CIELAB values (a*, b*, h*, L*, C*) and K/S for copper-treated undyed and copper-treated dyed fabric sample was determined using a reflectance spectrophotometer. Negative and positive values of b* indicate the degree of the blueness and yellowness of the dye, respectively, whereas negative values of a* indicate the extent of the greenness and positive values the degree of its redness. Chroma is represented by C*, brightness by L* (values between 0 and 100, where 0 represents the pure black color and 100 pure white), and h* represents the hue angle (00–3600). K/S values of cotton-dyed cloth were estimated employing Kubelka-Munk Equation (equation 5).

$$K/S = (1 - R)^2/ 2R \quad (5)$$

Here, R indicates the percentage reflectance, K is the absorption coefficient and S is the scattering coefficient.

4.10 Assessment of dye levelness

Both visual and objective techniques were employed to evaluate the levelness of the modified reactive dye on the dyed fabric. Visual examination involved observing the fabric from different angles and assigning grades from 1 to 5, with 5 indicating excellent levelness and 1 indicating poor levelness. To achieve more precise results, an objective method was also used. In this method, the fabric was scanned at 12 different points using a reflectance spectrophotometer, and K/S values were calculated. The calculated standard deviation for each K/S measurement was used to assess dye levelness. Lower standard deviation values corresponded to higher dye levelness; values within the range of 0.20 indicated excellent dye levelness, while values greater than 1.0 indicated poor dye levelness.

4.11. Evaluation of Comfort Properties

Air permeability is defined as the rate of air flowing perpendicularly through a known specific area. The flow of air is maintained under a prescribed air pressure differential between the two surfaces of a material. The test was performed as per ISO9237 by using an SDL air permeability tester. The air pressure difference between the two surfaces of the substrate was 100 Pa. Furthermore, the stiffness of untreated and treated with cuprous oxide particles cotton fabric samples was measured by TH 4 bending rigidity tester.

4.12 Assessment of antibacterial properties

The antibacterial activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements.

4.12.1. Zone of inhibition test (qualitative measurements)

Preparation of bacterial strain. The bacterial strains used in this study, *Escherichia coli* (Gram-negative, CCM 3954) and *Staphylococcus aureus* (Gram-positive, CCM 3953), were sourced from the Czech Collection of Microorganisms at Masaryk University in Brno, Czech Republic. Fresh bacterial suspensions were prepared by growing single colonies overnight at 37°C in nutrient broth. The turbidity of the bacterial samples was adjusted to an optical density of 0.1 at 600 nm (OD600) before conducting antibacterial experiments. Agar plates were prepared freshly for each antibacterial test. A sterilized cotton swab was immersed in the bacterial culture suspension and evenly spread across the agar plates. These plates were immediately used for the antibacterial activity assessments.

Determining Zone of Inhibition. The antibacterial activity assessment was conducted following the procedure described in detail in references [17][96]. For this study, squares of cuprous oxide particles coated cotton fabric measuring 6 x 6 mm were placed directly onto agar plates that had been inoculated with bacteria. Concurrently, untreated virgin cotton fabric was used as a control. The samples and inoculated agar plates were then incubated at 37°C for 24 hours. The zone of inhibition (ZOI) was calculated as the combined diameter (mm) of the cuprous oxide particles coated textile sample and the clear halo zone where bacterial growth was impeded. All measurements were performed in triplicate to ensure accuracy and reliability.

4.12.2. Reduction factor (quantitative measurements)

ISO 20743:2013 transfer method was used for the quantitative antibacterial analysis of dyed cotton. Agar plates were prepared and inoculated with 1 mL of inoculum as mentioned in the method. The control (2 specimens) and dyed samples (2 specimens) of 3×8 cm in dimensions were placed on prepared agar plates and samples were pressed down by applying 200 g weight. One specimen of each sample was detached from the agar plates and was placed on separate Petri dishes keeping the transferred surface faced upside. The samples were then incubated at 37^{o}C for 24 h. The second specimen of control and dyed samples were transferred immediately to 2 reagent bottles separately containing 30 mL of saline solution (0.85% NaCl) to get bacterial colony counts at 0 hr. After shaking bottles for 15 minutes, 8 serial dilutions of this saline solution were prepared and plating of all dilutions was performed on agar growth media as described in ISO 20743. The same procedure was repeated for samples placed in an incubator for 24h to get bacterial colony count after 24 h. The antibacterial activity (A) of the dyed cotton sample was determined using the formula given in Equation 6. Each sample was performed in triplicate for confirmation of results.

$$A=F-G \quad (6)$$

Where $F= (\log C_t - \log C_0)$ and C_0& Ct is the bacterial count of control cotton fabric at 0 and 24 h and $G= (\log T_t - \log T_0)$ and T_0 & Tt is the bacterial count of control fabric at 0 and 24 h.

4.13 Weight Gain

During the Cu_2O deposition, the weight gain percentage was examined according to the following equation:

$$w = \frac{m-m_0}{m_0} \times 100 \quad (7)$$

Where m is the final mass, m_0 is the original mass of the substrate and w is the total weight gain percentage.

4.14 Antifungal activity assessments

The antifungal property of the coated and dyed fabric sample was assessed using the AATCC 100-2004 standard testing method. A. Niger, a fungus species, was used for this test. Equation 10 was used to determine the antifungal effectiveness in terms of the percent change.

$$\text{Percentage reduction } R(\%) = \frac{(A-B)}{A} * 100 \quad (8)$$

Here, A and B indicate the number of spores for untreated control and treated cotton fabric samples, respectively.

4.15 Antiviral activity

The determination of virus titer reduction from the initial viral titer of infectivity (107) titer was done using Behrens and Karber's method. Vero-E6 cultures were maintained in Dulbecco's Modified Eagle Medium (DMEM), which contained 2% penicillin-streptomycin and 9% fetal-bovine serum (FBS) (PSA). Vero-E6 cultures were infected with the coronavirus at a ratio of 1:3 in polyethylene pots, and virus strains developed after one day. The virucidal impact of created viral stocks was investigated under a microscope. 10% FBS was added to the cell line, which was then frozen at 90 °C. Moderate centrifugation was used to filter the supernatant for 30 minutes at 5 to 7 °C and 3700 rpm. The supernatant was used as the viral stocks in the experiment, and all macro residual was eliminated. Vero-E6 cell lines were deposited at a concentration of 2 x 10^5 in 96-well plates and cultured under normal conditions (24 hrs at 37^{o}C in 6% CO_2) to determine the virus titer. Each sample was diluted ten times from 10^1 to 10^8. Every dilution was injected into cell lines, where they were cultured for 3 days at 6% CO_2. The procedure established by Behrens and Kerber was used to measure the coronavirus titer in cultivated cell lines. Following that, 20×20 mm fabric sample vials were filled with the treated and control fabric samples. 100 µl of viral loads were passed through the treated and control fabrics, and any recoverable viral loads in containers were cleaned with the filter. There was a 10^1 to 10^8 dilution of the coronavirus. All serial dilutions were implanted into Vero-E6 cell lines, where they were then cultured for 3 days at 37 °C with 6% CO_2. The method of Behrens and Karber was employed to determine the Coronavirus titers in the cultivated cell lines.

4.16 Durability of bioactive fabrics

The durability of developed fabric samples was evaluated to check their stability in service. Fabrics were washed with ISO 105-C01. All fabric samples were mixed in a conventional detergent solution with a 50:1 liquor ratio. After that, samples were washed for 35 minutes at 40 °C at a 600-rpm speed. Fabrics were then dried and conditioned for a total of 24 hours under normal atmospheric conditions. Electrical conductivity, antibacterial findings, and SEM observations all supported durability.

5. RESULTS AND DISCUSSIONS

5.1 The Formation of Cu_2O particles (by using three different reducing agents) and Deposition on Cotton Fabric

The study describes the formation of cuprous oxide Cu_2O particles by the Fehling solution method. Three different types of reducing agents were used to make the particles. Subsequently, particles were deposited on cotton fabric. The effect of three different types of reducing agents was analyzed against the bioactive properties.

5.1.1 Morphology of Cu_2O particles coated cotton fabrics

5.1.1.1 SEM microstructure.

Scanning electron microscopy was employed to observe the deposition of cuprous oxide particles which were reduced by different reducing agents. Figure 25 shows nanometres scale images of the surface morphologies of cuprous oxide particles on the surface of cotton fabric. There was an obvious change in the size and surface morphology of cuprous oxide particles reduced by different reducing agents. It was noticed that the cuprous oxide particles which were reduced by glucose, had a big particle size as compared to the cuprous oxide particles reduced by ascorbic acid and Sodium hydrosulphite. While the comparatively smallest and even distribution of particles was observed in case of sodium hydrosulphite.

To keep the size of nanoparticles small, the initial concentration of salt and strength of the reducing agent plays an important role. The reason is that sodium hydrosulphite is the strongest and more compatible reducing agent for copper salts as compared to ascorbic acid and glucose [14][16]. The strong reducing agent provides the proper reduction of metal salt and formed the small nanoparticles. While the weak reducing agent (glucose) provided the improper reduction of copper salt and produced the agglomerated structures, which in turn cover the less surface of fiber as shown in Figure 25c. Less salt and a strong reducing agent provide more nucleation of salt and produce finer particles. The theory is further assisted by a study, where different types of reducing agents were used to form metal nanoparticles. The fine nanoparticles were created by the strongest reducing agent 3.8nm ($NaBH_4$) as compared to 4.3nm (N_2H_4), and 15.8nm ($C_6H_8O_6$) [97].

The cuprous oxide particles reduced by sodium hydrosulphite and ascorbic acid covered the complete fiber surface (Figures 3b and 3a). Figure 3a showed the continuous and uniform distribution of particles on the surface of cotton. Furthermore, the deposition was found more uniform and denser with the increase in the concentration of copper salts. This

trend was further justified by the particle size images of cuprous oxide particles as shown in Figures 25a, b, and c.

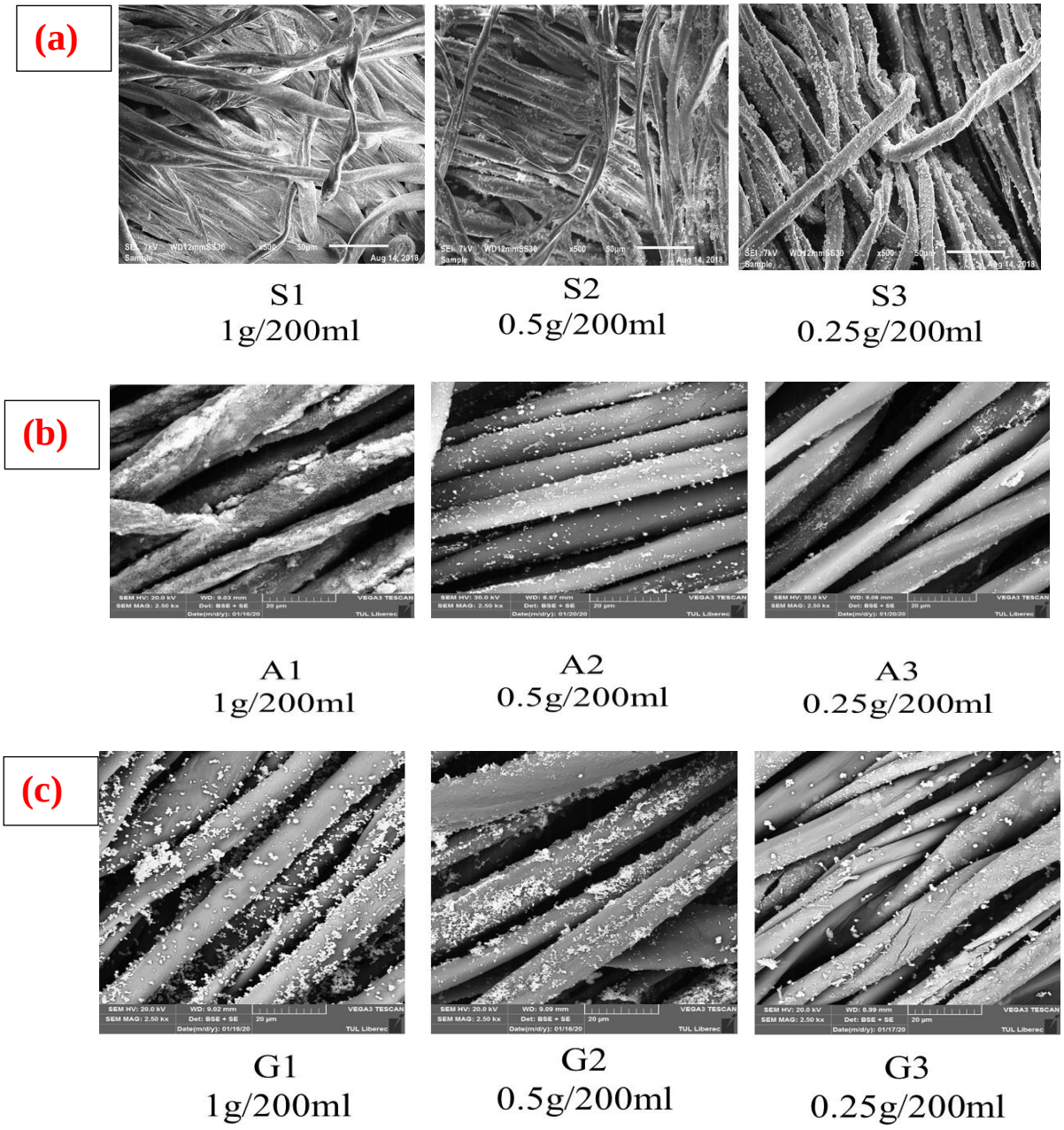
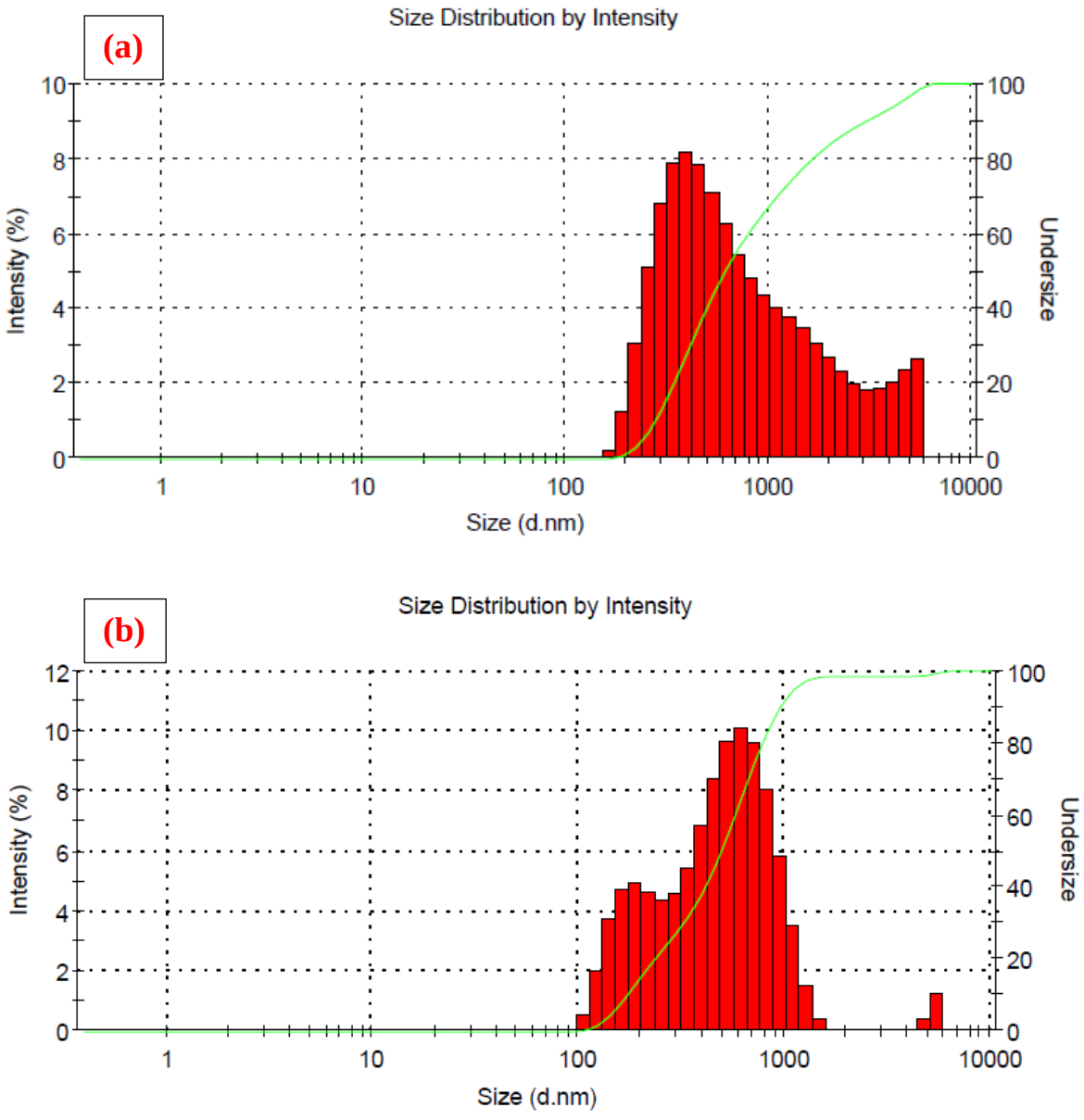


Figure 25: Surface morphology of cotton fabrics coated with cuprous oxide particles reduced by (a) Sodium hydrosulphite, (b) ascorbic acid, (c) glucose.

5.1.1.2 Particle size distribution

The particle size was measured by the dynamic light scattering technique based on Brownian motion. The average particle size distribution of cuprous oxide particles is shown in Figure 26. The cuprous oxide particles were found to have a multi-modal distribution with sizes varying from micrometers to the nanometers range. The average particle size of cuprous oxide particles reduced by glucose was about 900 nanometers, while the average particle size

of cuprous oxide particles reduced by ascorbic acid and sodium hydrosulphite was about 500 and 450 nanometers respectively.



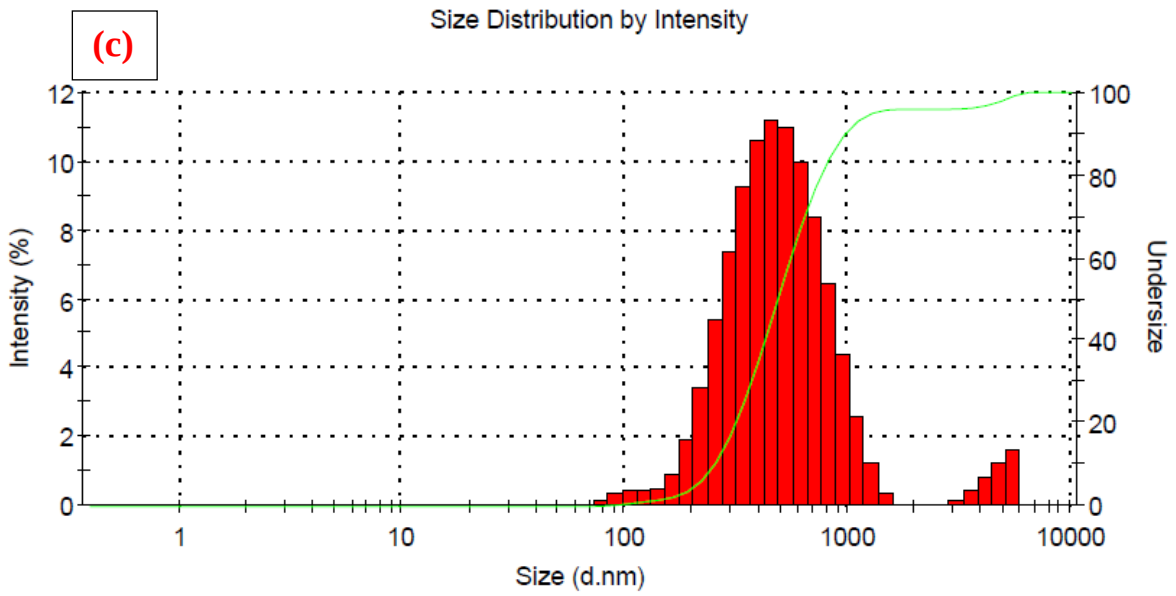


Figure 26: The average particle size distribution of cuprous oxide particles reduced by (a) glucose, (b) ascorbic acid, (c) Sodium hydrosulphite.

5.1.1.3 XRD analysis

The XRD analysis was carried out to know the phase composition of deposited cuprous oxide particles. Figure 27 shows the XRD patterns of samples for the 2θ range of 10 to 80 degrees with a step of 0.02 degrees. The phase purity of the prepared cuprous oxide particles can be seen from the perfect indexing of all the diffraction peaks to the cuprous oxide structure. The diffraction peaks observed at 2θ of 29.6 º, 36.5 º, 42.4 º, 52.1 º, 61.5 º, and 73.7 º represented (1 1 0), (1 1 1), (2 0 0), (2 1 1), (2 2 0) and (3 1 1), reflections respectively [98][99]. The crystalline nature of cuprous oxide particles was confirmed by the sharpness of the peaks, whereas the broadening of the peaks justified the formation of nanoscale cuprous oxide particles. As such no characteristic peaks of impurities were detected, except the peak of copper oxide phase at 2θ of 38º and 78º as shown in Figure 27 [100][101].

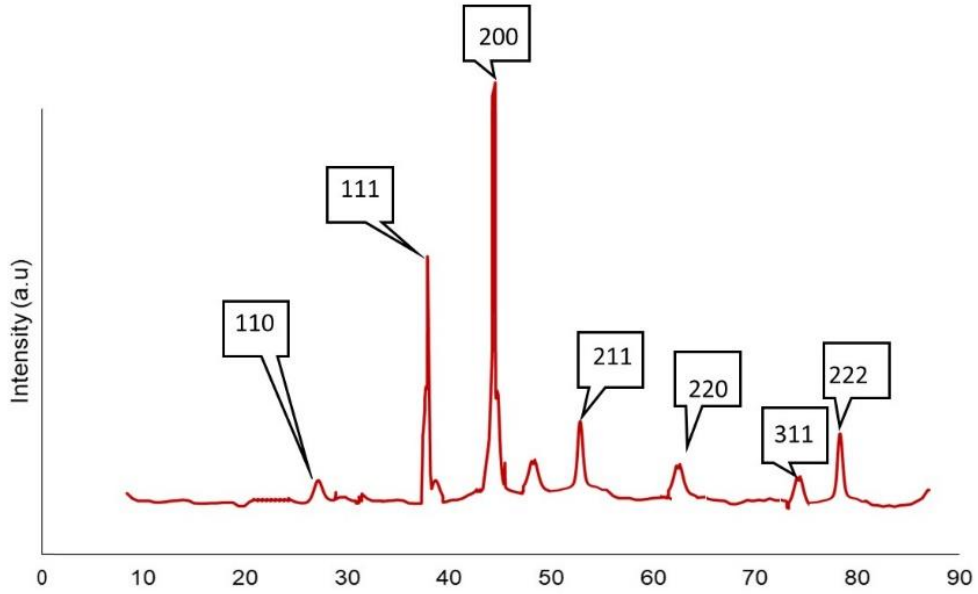


Figure 27:XRD patterns of cuprous oxide particles.

5.1.2 Antibacterial activity of the cuprous oxide nanoparticle-coated fabrics

The antibacterial activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements.

5.1.2.1 Zone of inhibition test (qualitative measurements)

The zone of inhibition test is a type of qualitative measurement. The test was performed against both types of bacteria (Gram-negative *E. coli* and Gram-positive *S. aureus*). Figures 28 and 29 show the clear zones of inhibition around all fabric samples after 24 h of incubation in the dark at 37 °C. The zone of inhibition was less for the cuprous oxide-coated fabrics reduced by glucose. The cuprous oxide-coated fabrics reduced by sodium hydrosulphite showed the most significant antibacterial zone to *E. coli and S. aureus*. The zone of inhibition test was repeated three times for each sample and the average value against each reducing agent is presented in Figures 6, 7, and 8. These results showed that the deposited cuprous oxide particles present strong sterilization to the *E. coli* and *S. aureus* due to the free-standing of the particles. However, *Staphylococcus* aureus depicted the highest sensitivity as compared to *Escherichia coli.* The zone of inhibitions for *Staphylococcus aureus* increased from 5 to 7 mm, while for Escherichia coli it increased from 4 to 6 mm with the increase in sodium hydrosulphite concentration. What is noteworthy is that the annulus of the inhibition zone increases with the increase in the concentration of the reducing agent. In other words, the prepared fibers with more cuprous oxide particles with an increase in the concentration of reducing agents. This is indicating that more cuprous oxide contents will influence the antibacterial activity, which is similar to the previous study [7].

The antibacterial property of coated fabrics can be attributed to the combination of chemical and physical interactions of bacteria with copper particles. The cuprous oxide nanoparticles can incorporate into the cell via endocytic mechanisms. Afterward, the cellular uptake of ions increased as ionic species were subsequently released within the cells by nanoparticle dissolution [102]. This resulted in high intracellular concentration gained within the cell for further massive oxidative stress. The mechanisms associated with the antibacterial behavior of copper nanoparticles can be summarised as shown in Figure 28.

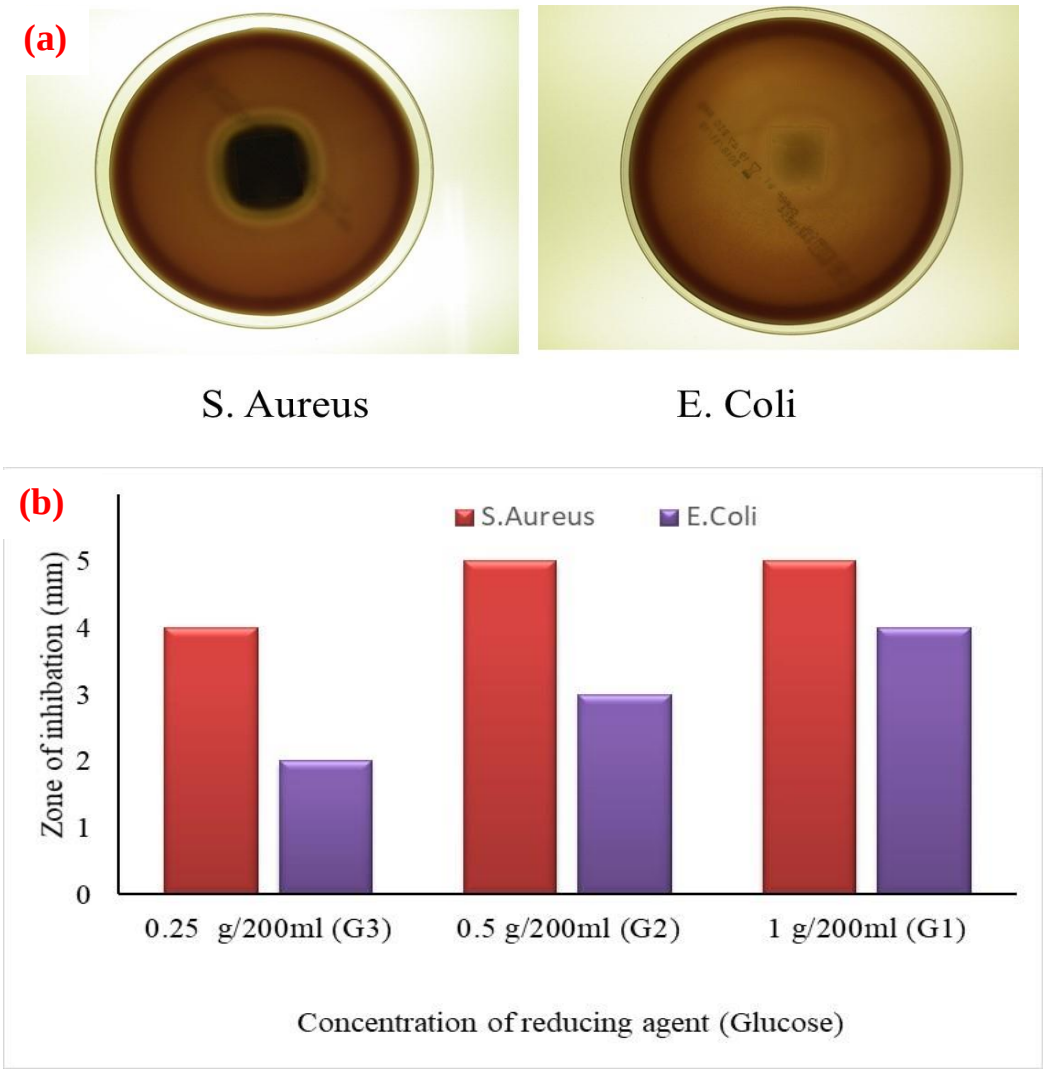


Figure 28: (a) Image of the zone of inhibition (b) the average value of the zone of inhibition against each concentration of the glucose-reducing agent.

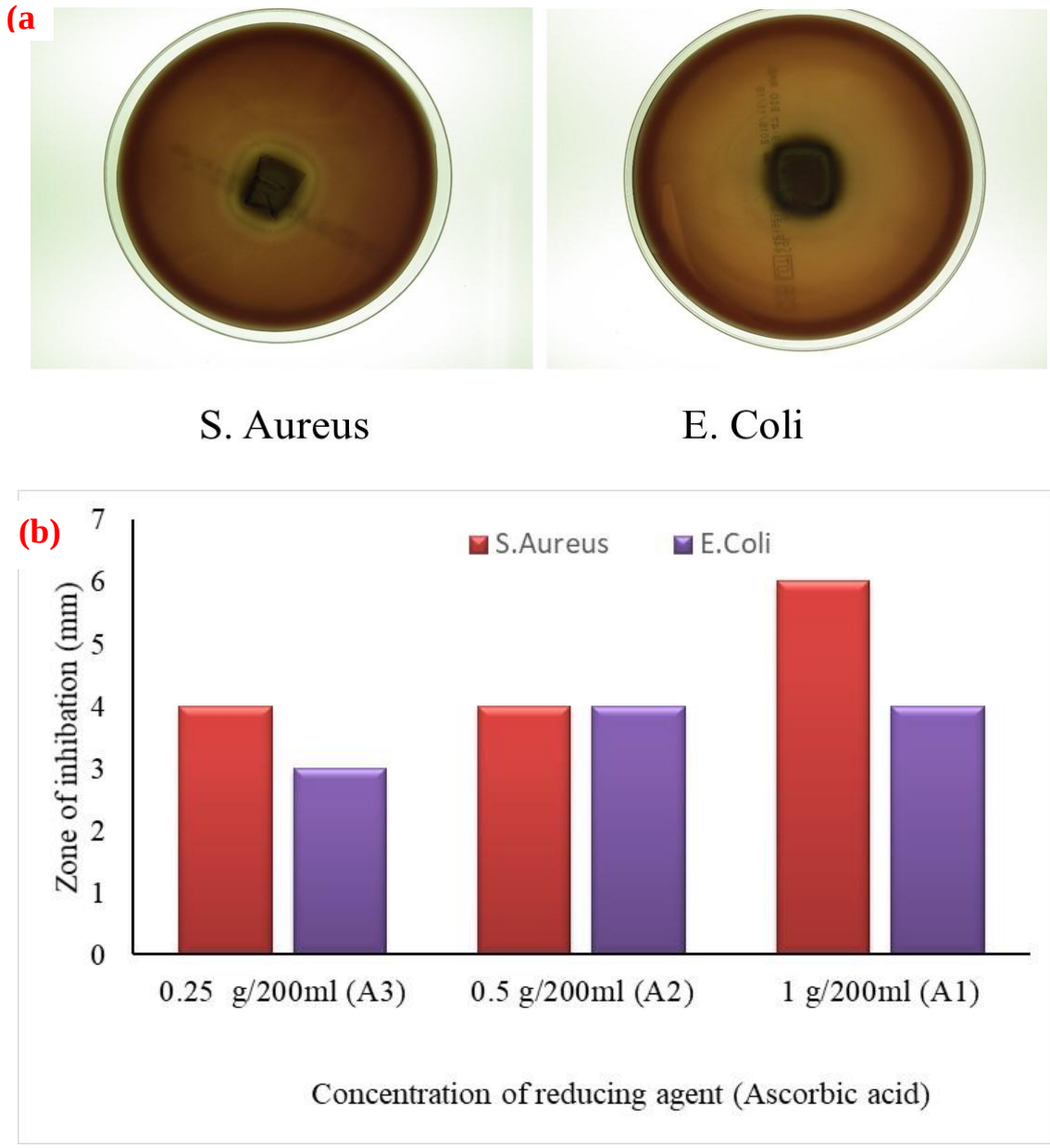


Figure 29:(a) Image of the zone of inhibition (b) the average value of zone of inhibition against each concentration of Ascorbic acid reducing agent.

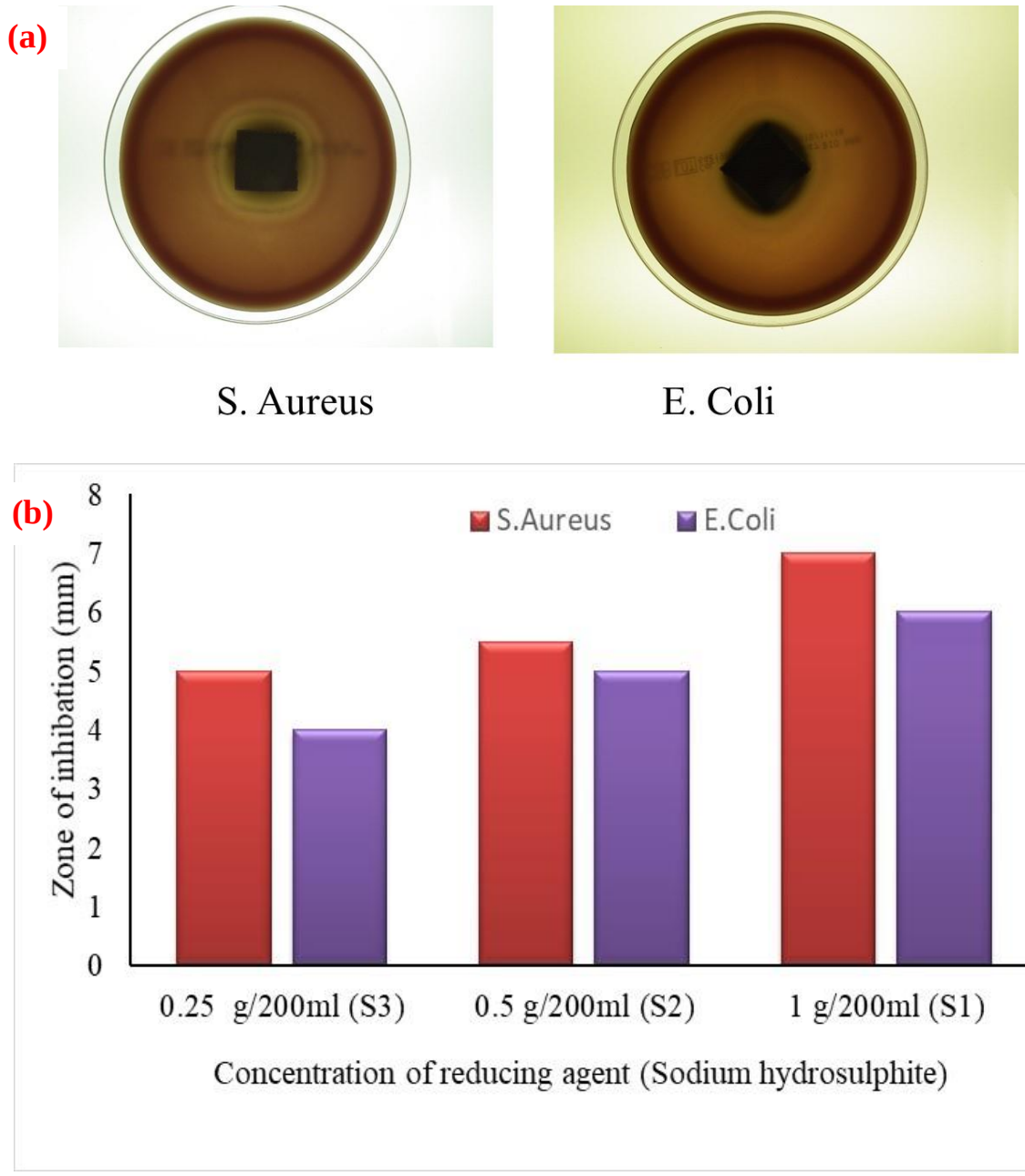


Figure 30:(a) Image of the zone of inhibition (b) the average value of zone of inhibition against each concentration of sodium hydrosulphite reducing agent.

5.1.2.2 Reduction factor (quantitative test)

AATCC test method 100-2004 was adopted for quantitative measurements. This method is based on the reduction (in percent) of the inoculated concentration of the bacteria due to the effect of the sample. The result is many survivor bacteria colonies (CFU) and from this number, there is calculated inhibition degree (in %). The reduction percentage of all samples (untreated, reduced with glucose, ascorbic acid, and sodium hydrosulphite) is given in Table 6. There was no reduction percentage for the untreated sample, while all other

samples showed a good reduction percentage against both types of bacteria (gram-positive and negative) [103][104].

Table 6: The reduction (in percent) of the inoculated concentration of the bacteria due to the effect of the cuprous oxide-coated sample.

Sample	Escherichia coli	Staphylococcus aureus
	The result, % inhibition	The result, % inhibition
Untreated standard	0 %	0 %
Glucose G1	99.99%	99.99%
G2	90.9%	99.99%
G3	28.2%	99.99%
Ascorbic Acid A1	99.99%	99.99%
A2	99.99%	99.99%
A3	93.3%	99.99%
Sodium hydrosulphite S1	99.99%	94.7%
S2	99.99%	99.99%
S3	99.99%	99.99%

For a better overview, the log CFU / ml concentration was calculated and the results were plotted. Where the concentration of survival of bacteria was found against both bacteria (S. aureus and E. coli) and their respective graphs are shown in Figures 31 and 32. The untreated sample showed a lot of survival colonies because of no effectiveness against bacteria, while compared to the standard, all other samples (against bacterial strains of E. coli and S. aureus) showed good inhibition and a smaller number of survival colonies. Furthermore, there is a remarkable reduction in the concentration of survival colonies in case of cuprous oxide particles reduced by sodium hydrosulphite.

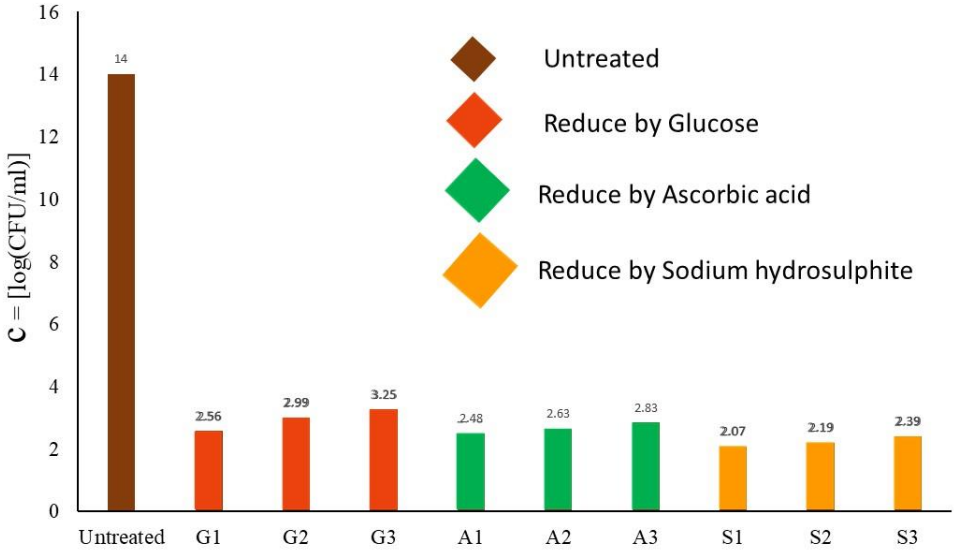


Figure 31: The concentration of survival of bacteria (S. aureus) against cuprous oxide coated fabrics reduced with different reducing agents.

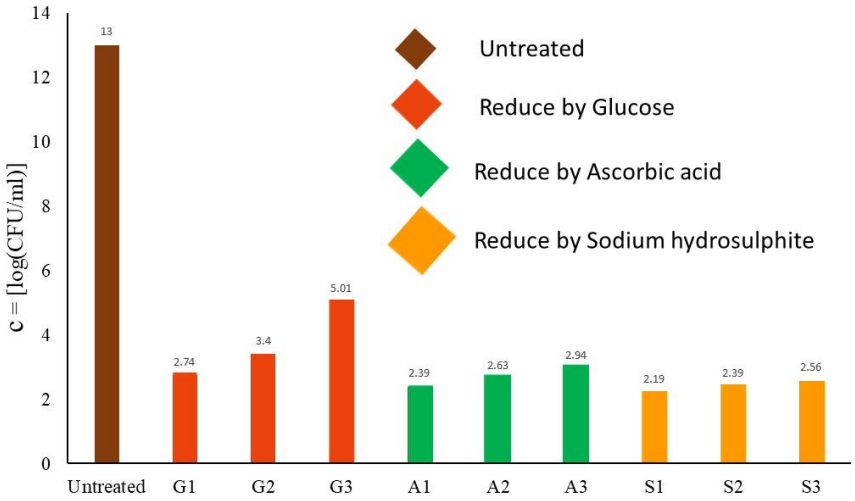
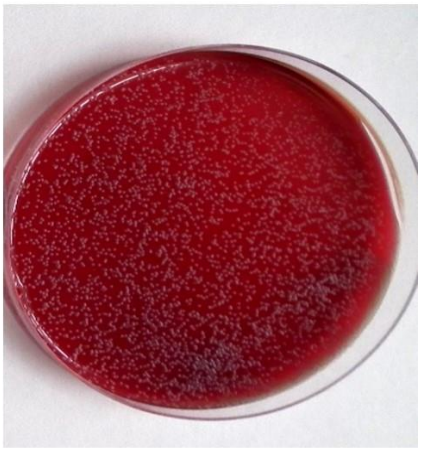


Figure 32: The concentration of survival of bacteria (E. colie) against cuprous oxide coated fabrics reduced with different reducing agents.

The trend was further justified by selected images of the concentration of bacterial growth for the untreated (pristine) cotton fabric and treated (G1, A1, and S1) samples as shown in Figure 33. The untreated sample remained ineffective against bacterial growth, while the treated fabrics coated with cuprous oxide particles showed clear effectiveness against bacterial growth. However, with a further increase in cuprous oxide particles loading at higher concentrations of sodium hydrosulphide (up to 1g/200ml) there was a significant improvement in colony reduction was observed with an efficiency greater than 99% for both types of bacteria.

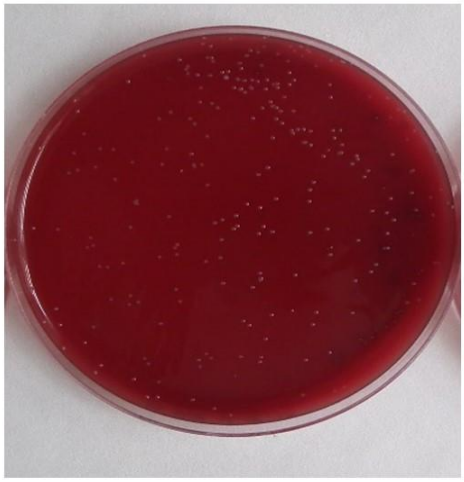
(a) Untreated cotton fabric

E. Colie

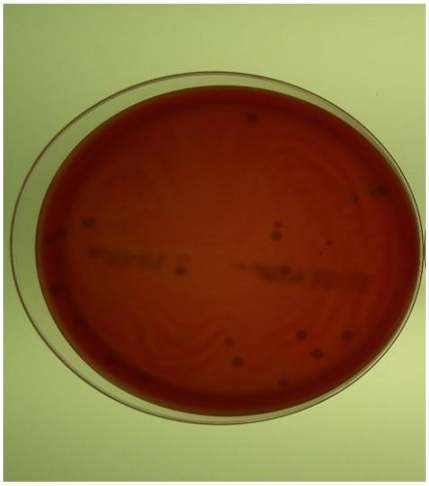


S. aureus

(b) cuprous oxide particles reduced by glucose



E. Colie



S. aureus

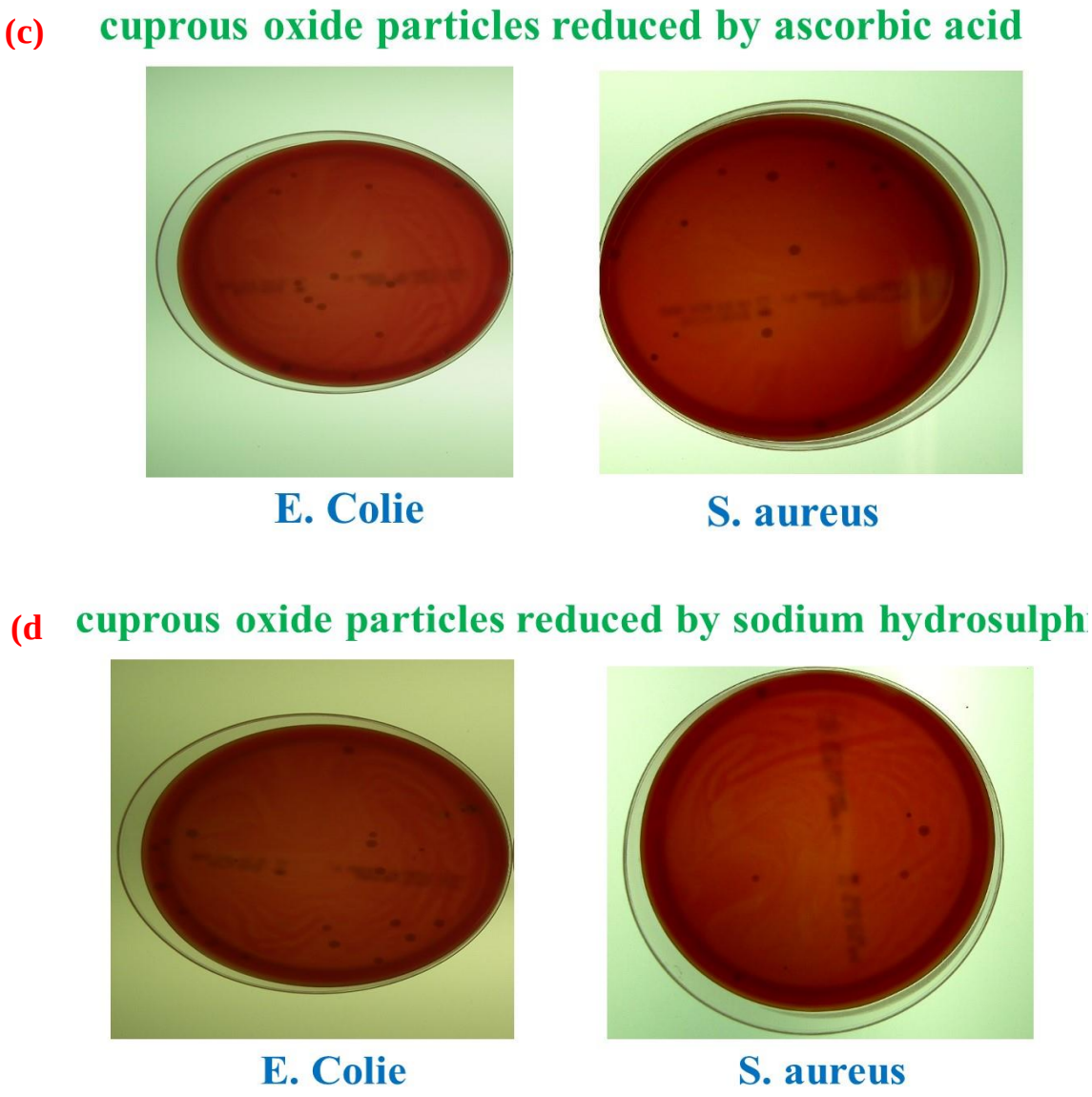


Figure 33: Images of concentration of bacterial growth for the (a) untreated (pristine) cotton fabric, (b) for the G1 fabric sample, (c) for the A1 fabric sample, and (d) S1 fabric sample.

5.1.3 Weight gain percentage and antimicrobial effect

The percentage of fabric weight gain was measured with an increase in the concentration of cuprous oxide particles. The effect of weight gain against antimicrobial properties was measured for all samples and their respective graphs are shown in Figures 34 (a, b, and c). From the trend lines, it is clear that with the increase in the concentration of cuprous oxide particles, the mass gain was going to increase and the antimicrobial effect (zone of inhibition) were significantly increased. The maximum antimicrobial effect (zone of inhibition) and maximum weight gain percentage values were confirmed at 1.25 g/200ml of all reducing agents. The mass gain percentage and antimicrobial effect of cuprous oxide

particles reduced with sodium hydrosulfide is higher than all, while the mass gain percentage and antimicrobial effect of cuprous oxide particles reduced with glucose is less than all.

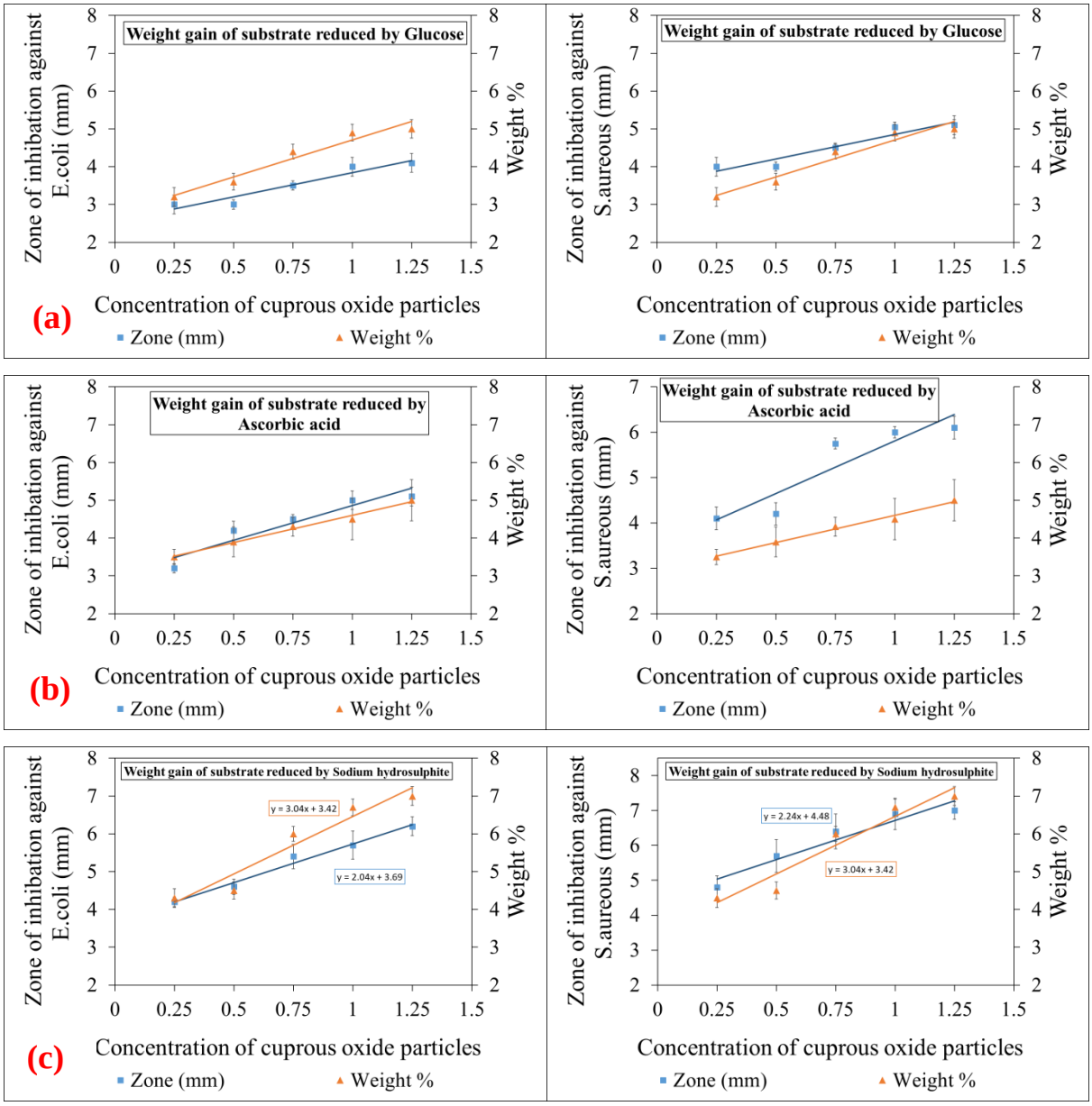


Figure 34: The effect of weight gain against antimicrobial properties for fabric samples (a) reduced by glucose, (b) reduced by ascorbic acid, and (c) reduced by sodium hydrosulphide.

5.1.4 Thermo-physiological comfort properties

The vision behind wearable antimicrobial textiles forecasts future hygienic systems to be an integral part of our everyday outfits. Such hygienic textiles have to meet special requirements regarding wearability. When we talk about wearable antibacterial fabrics, then comfort is the real parameter that we cannot avoid. Air permeability and stiffness are the most important comfort properties for wearable antibacterial textiles.

5.1.4.1 Air permeability

Air permeability is an important parameter for textiles and maintains thermal comfort. It helps to exchange air when heat and perspiration are generated from the body [109]. The results of fabric air permeability are shown in Figure 35. The air permeability results were found for all types of fabrics (treated and untreated).

From the results, it is clear that the application of very fine cuprous oxide nanoparticles to the fabrics has very little effect on air permeability. The air permeability of the untreated fabric is about 130 mm/s while the air permeability for all other cuprous oxide nanoparticles coated fabrics is in the range of 123 to 126 mm/s. Showing that there is a minor decrease in air permeability even after depositing the cuprous oxide nanoparticles. There are two factors responsible for this phenomenon. Firstly, there may be a relaxation shrinkage in the fabric structure due to dipping cuprous oxide nanoparticles solution, causing the yarns to come close and hinder the flow of air. Secondly, during the application of nanoparticles, have deposited on the yarn structure and interstices, reducing the fabric air gap spacing (pore size). The fabric pore size has a direct relation with the air permeability of the fabric. Therefore, a reduction in pore size has caused a decrease in the air permeability of the fabric. The spun yarns of cotton have a low uniformity and hairiness, which cause resistance to the flow of air and lead to low air permeability. It is the beauty of nanoparticles that they will cover more surface area over the yarn but will not entrap in the spaces between the fabric structures.

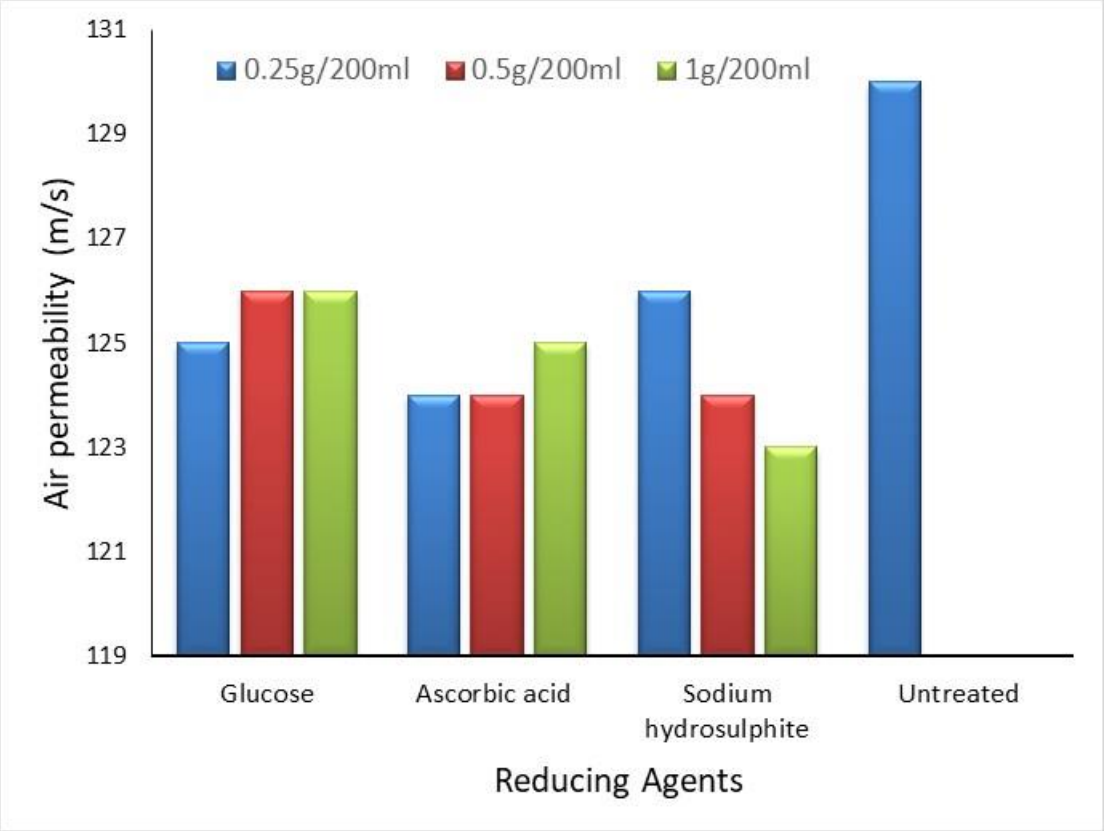


Figure 35: The air permeability results for all types of fabrics.

5.1.5 Stiffness

The term stiffness of fabric describes its ability to resist deformation and keep standing without support. This property is important regarding comfort and desirable draping. Stiffness can be calculated from bending length and flexural rigidity. The stiffness of coated and uncoated fabric samples was found and average values are given in Figure 36. Fabric stiffness was found to increase with the increase in the concentration of reducing agents. It means that an increase in the concentration of reducing agents causes to increase in the deposition of cuprous oxide particles. The reason is that coating increased inter-fiber friction and abrasion at fiber crossover points [110]. However, the effect of the increase in rigidity overall is insignificant.

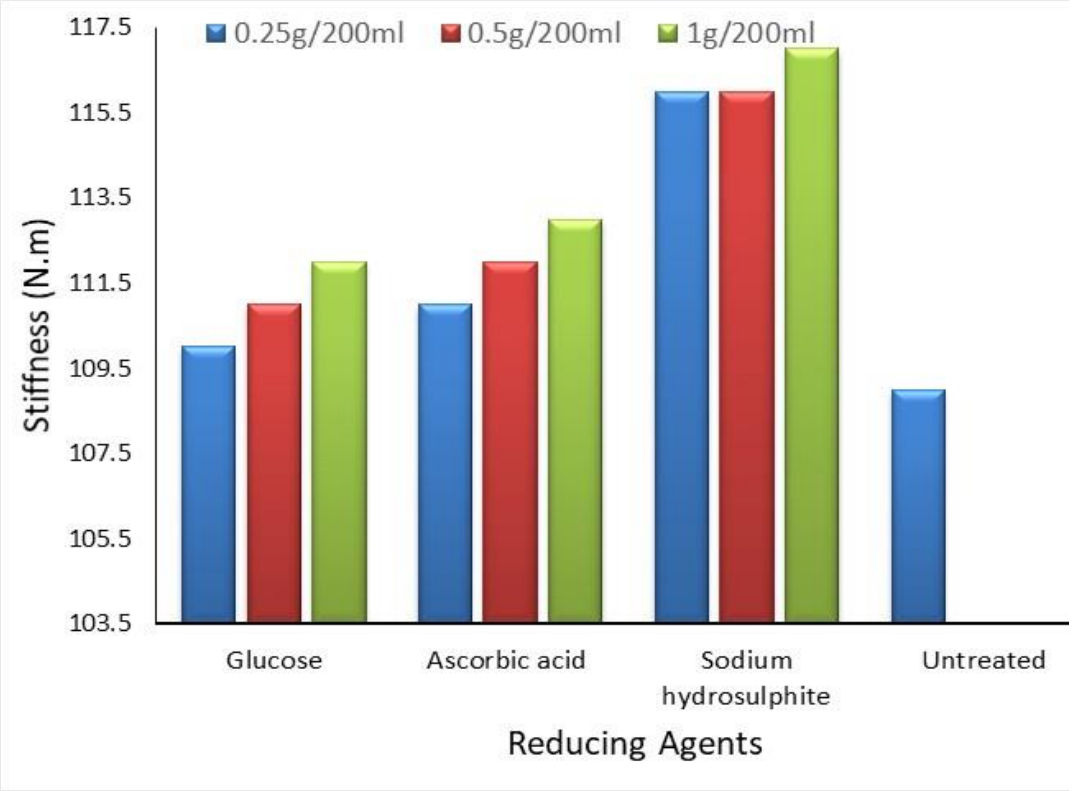


Figure 36: The stiffness of coated and uncoated fabric samples.

5.1.6 Durability of cuprous oxide coated fabrics

As mentioned earlier, the cuprous oxide particles were attached to the surface of fabrics through the combination of various bonding. The additional cuprous oxide particles filled the gaps and interspaces between microfibers and stacked them together to form hygienic antibacterial networks. This behavior of absorbance and adherence was further described by the durability of the antibacterial effect against washing in water. The functionalized fabrics were also soaked, squeezed, and twisted in water. The cuprous oxide-coated samples showed good washing properties without peeling off and precipitating in the water. Later on, an adhesion test was performed with transparent tape. The tape remained transparent i.e. no visible particles were observed on the tape. Hence indicating the robust interactions and reasonable mechanical adhesion properties among hygienic cuprous oxide particles and fabric.

To investigate these properties, the antibacterial properties of all fabrics were investigated after washing. The samples were washed according to the standard washing test method ISO 105-C01. The antimicrobial values of all developed samples before and after washing are given in Table 7.

It is clear from the given values that there is almost a 50% decrease in the zone of inhibition

after washing. This shows that prepared samples are effective against pathogens even after severe washing. Furthermore, the trend was also justified by the SEM analysis after washing (for the samples G1, A1, and S1) as shown in Figure 37. The retention of particles over the surface of the fabric reinforced the fact that particles are firmly attached to the fibers and interspaces.

Table 7: Antimicrobial properties of Cu_2O coated fabrics after washing

No of samples	**Code**	**ZOI (mm) E. Colie**	**ZOI (mm) S. Aureus**	**ZOI (mm) E. Colie**	**ZOI (mm) S. Aureus**
		Before washing		**After washing**	
1	G1	4	5	2	3
2	G2	3	5	1	2
3	G3	2	4	0	3
4	A1	4	6	3	4
5	A2	4	4	3	2
6	A3	3	4	2	2
7	S1	6	7	4	4
8	S2	5	5	4	3
9	S3	4	5	2	3

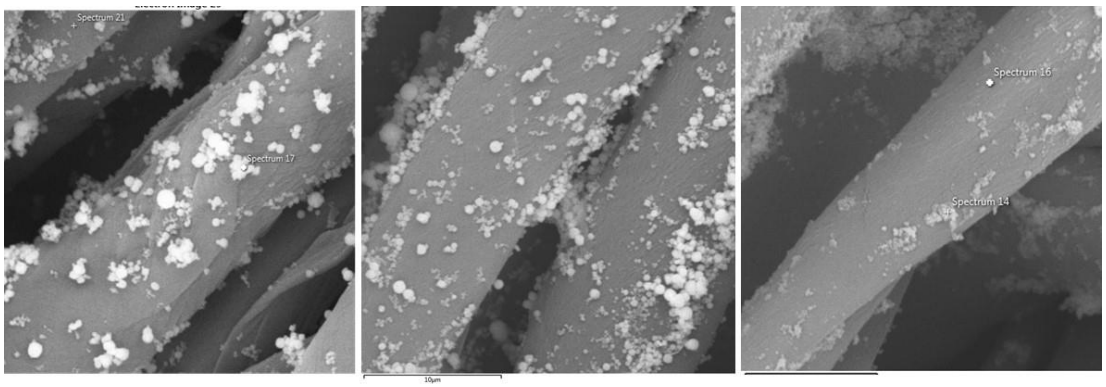


Figure 37: SEM analysis after washing (for the samples G1, A1, and S1)

5.2. Copper-treated environmentally friendly antipathogenic cotton fabric with modified reactive blue 4 dye to improve antibacterial and aesthetic properties

The objectives of this part were to develop an environmentally friendly, low-price, easy, and fast method for developing antipathogenic (antibacterial, antifungal, and antiviral) cuprous oxide-coated multifunctional fabrics. At first, fabrics were sensitized with citric acid then the formation of Cu_2O particles was done by the Fehling solution method. Then, the cuprous oxide particles were deposited on cotton fabrics. Three different types of reducing agents with different concentrations were selected to make the Cu_2O particles. Surface morphology and presence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. In the second step, a reactive antibacterial dye was made (by reacting Reactive Blue 4 with triclosan). The molecular structure of the modified dye was confirmed through FTIR and ^{13}C-NMR. The details about structure of dyestuff, triclosan modification, dyeing procedure and reactivity of modified dye is already described in previous sections 4.4 and 4.5.

The resultant antibacterial dye was applied on copper-treated cotton fabrics through exhaust dyeing protocol. The dyed fabric was characterized through colorimetric data (L*, a*, b*, C, H, and K/S), levelness of dye, fastness properties as well as exhaustion and fixation rates. The antipathogenic activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements. The strongest antipathogenic effect was found for the fabrics coated with cuprous oxide particles reduced with sodium hydrosulphite at 1g/L. Furthermore, the utility of hygienic antimicrobial-developed fabrics was analyzed for comfort properties regarding air permeability and stiffness. In the end, the durability of the coating was confirmed by measuring the antibacterial properties and SEM analysis after washing.

5.2.1 FTIR Analysis

FTIR Peaks (KBr): 3323 cm^{-1} (amine -NH_2, -NH stretch), 3378 cm^{-1} (amine -NH_2, N-H stretching), 1046 cm^{-1} (C-O-C ether linkage stretch), 638 cm^{-1} (C-Cl stretch). The FTIR spectra of the unmodified dye (Reactive Blue 4) and modified dye (modified with an antibacterial agent) has shown in Figure 38. The spectra successfully confirmed that an antibacterial agent (triclosan) has been successfully incorporated into the structure of the dye through a covalent bond. It was confirmed by the appearance of a new sharp peak of strong intensity at 1046 cm^{-1}. This sharp peak is a characteristic peak of ether linkage (C-O-C) [111]. The formation of the covalent bond between the hydroxyl group of triclosan and chlorine of the triazine ring has resulted in the development of ether linkage (C-O-C). Therefore, the absence of a sharp and strong peak at 1046 cm^{-1} in unmodified dye and the

appearance of this peak in the spectra of modified dye confirmed that modification of dye with the antibacterial agent has been successfully achieved.

The modification of the dye was further supported by the increase in the intensity of the peak that occurred at 638 cm^{-1}. This peak is also present in the spectrum of unmodified dye and is attributed to the C-Cl stretching vibrations [112]. However, the intensity of the same peak is significantly increased in the spectrum of modified dye. The increase in peak intensity could be due to the increase in the C-Cl linkages in the structure of the modified dye because three C-Cl linkages are present in the structure of triclosan which further confirmed that triclosan is embedded in the structure of the dye molecule.

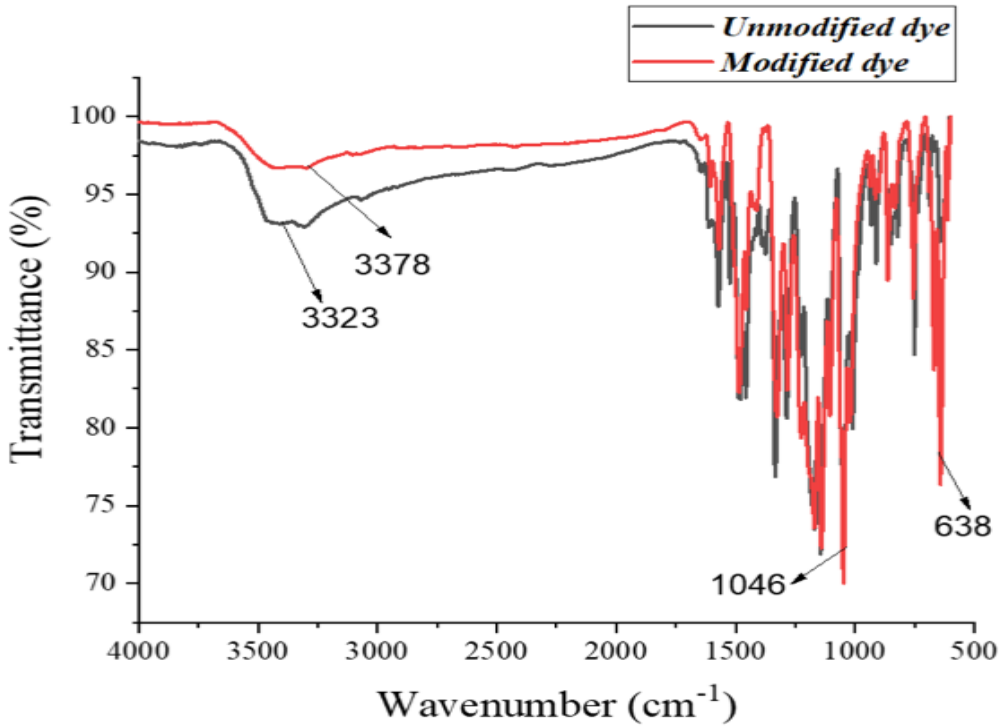


Figure 38: IR spectra of modified and unmodified dye.

5.2.2 NMR Analysis of modified functional reactive dye

^{13}C-NMR (600 MHz, D_2O): δ 32.9. ppm (s), 38.1 ppm (s), 121.4 ppm (s), 128.3 ppm (s), 150.3 ppm (s), 153.6 ppm (s), 161.3 ppm (s), 163.1 ppm (s). Figure 39 shows the ^{13}C - NMR spectrum for the modified Reactive Blue 4 dye. Two sharp up-field singlets occurred at 32.9 and 38.1 ppm could be attributed to the presence of 2 saturated carbon atoms present in the central cycloalkane ring in the anthracene moiety of the dye [113]. Meanwhile, the existence of two sharp peaks that occurred at 121.4 and 128.3 ppm could be due to the presence of a benzene ring in an unmodified dye structure [114]. Whereas, the occurrence of two other sharp singlets at 150.3 and 153.6 pm could be ascribed to the two benzene rings of

triclosan attached through oxygen atom [115]. The presence of these two peaks confirmed that an antibacterial agent (triclosan) has successfully incorporated into the structure of reactive blue 4 dye through covalent linkage. Furthermore, the presence of two sharp downfield singlet at 161.3 and 163.1 ppm could be a result of the triazine ring present in the structure of unmodified dye [116].

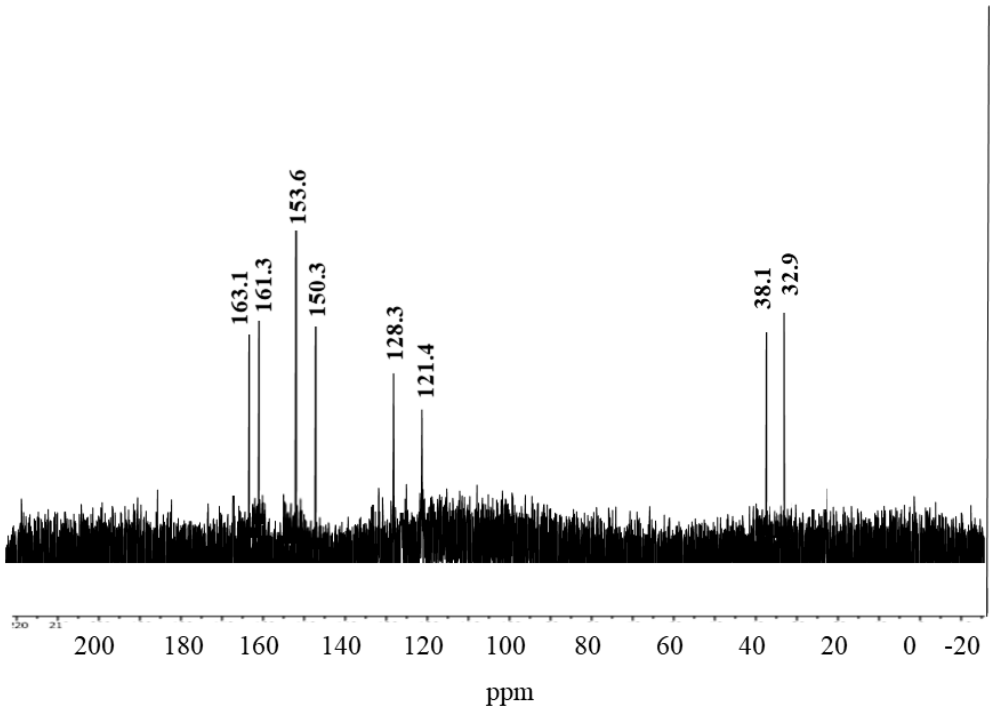


Figure 39: 13C-NMR spectra for modified Reactive Blue 4 dye.

5.2.3 Colorimetric data measurement

The colorimetric data for the copper-treated undyed and dyed fabric samples were evaluated and obtained results are given in Table 8. There was a significant difference between the colorimetric data of copper-treated undyed and dyed fabrics. The K/S value for the dyed cotton fabric was higher (12.13) as compared to undyed fabric (7.08) which showed that the application of dye has changed the light-colored fabric to comparatively dark-colored fabric. The dark shade for the dyed fabric samples was further confirmed by the difference in L* values of both fabrics. The L* value for the dyed fabric sample was lower (39.14) than the L* value of the undyed fabric sample (52.43) which showed that dyed fabric has more dark shade when compared to the undyed fabric. The chroma (C*) value for the dyed sample was also lower (19.14) than for undyed fabric (32.87) which suggested a brighter shade for undyed fabric samples and a darker and duller shade for dyed fabric samples. The a* and b* values for the undyed fabric samples were positive which indicated a reddish and yellowish

shade for the undyed fabric whereas both a* and b* values were negative for dyed fabric samples indicating greenish and bluish shade for the dyed fabric samples.

Table 8: Colorimetric data for copper treated undyed and dyed fabric samples.

Sr.#	Properties	Copper-coated fabrics	Copper-coated dyed fabrics
1.	Fabric color		
2.	K/S	7.08	12.13
3.	L*	52.43	39.14
4.	a*	5.15	-9.34
5.	b*	31.56	-16.13
6.	C*	32.87	19.14
7.	H*	80.15	241.13

5.2.4 Levelness of Copper treated undyed and dyed fabric

The color levelness effect was assessed to determine the uniformity of appearance in the dyed fabric. To achieve this, a reflectance spectrophotometer was employed to scan both the undyed copper-coated and dyed copper-coated cotton fabric, and K/S values were collected from 12 distinct points. The obtained K/S values and their corresponding standard deviations are presented in Table 9.

For the dyed copper-coated fabric, the calculated standard deviation was 0.24, indicating excellent levelness properties. This result suggests that the dye is uniformly distributed across the surface of the fabric, leading to an even and consistent appearance. In contrast, the undyed copper-coated fabric exhibited a significantly higher standard deviation of 0.81, highlighting the uneven and inconsistent appearance of the fabric.

The outcomes of the dye levelness assessment affirm that the dyed copper-coated fabric achieves a smooth and uniform appearance compared to the undyed copper-coated fabric, aligning with one of the objectives of the study. In the visual evaluation, the dyed copper-coated fabric was awarded a grade of 5 (excellent levelness), while the undyed copper-coated fabric received a grade of 3 (moderate levelness). This visual evaluation further supports the aforementioned findings, emphasizing that the application of dye on the copper-coated fabric results in an even and harmonious appearance of the fabric.

Table 9: Reflectance measurement data for the undyed and dyed copper-coated fabrics.

Number of	K/S Values	Standard	K/S Values	Standard deviation

Scans	Undyed sample	deviation (S.D)	Dyed sample	(S.D)
Scan 1	7.08	0.81	12.35	0.24
Scan 2	8.65		13.17	
Scan 3	9.01		12.55	
Scan 4	7.34		12.73	
Scan 5	8.47		13.11	
Scan 6	9.34		12.78	
Scan 7	7.56		12.78	
Scan 8	7.38		12.77	
Scan 9	8.39		12.65	
Scan 10	9.01		12.63	

5.2.5 Fastness properties of copper-coated dyed fabric

The exhaustion, fixation, washing, rubbing, and light fastness properties of the three samples—DG, DA, and DS—were evaluated according to standard testing methods, and the obtained results are presented in Table 5. All dyed samples demonstrated favorable washing fastness ratings ranging from 3 to 5, excellent light fastness ratings of 4 to 5, and good rubbing fastness rating of 4.

The exhaustion and fixation rates of the dye for all samples were notably high, with a dye exhaustion rate of 96% and a fixation rate exceeding 89%. These remarkable fastness properties and high exhaustion and fixation rates can be attributed to the fiber-reactive nature of the employed reactive dye. Reactive dyes have the capability to establish strong covalent bonds with the hydroxyl groups present in cotton fabric. This inherent property contributes to achieving excellent dye fixation and fastness properties.

Overall, the dyed samples, namely DG, DA, and DS, exhibit impressive performance in terms of fastness properties and dye exhaustion/fixation rates, underscoring the effectiveness of the chosen reactive dye and dyeing procedure.

Table 10: Exhaustion, fixation, and fastness (washing, rubbing, and light) of dyed fabric.

Sr. #	Sample Code	Exhaustion %	Fixation %	Washing fastness	Rubbing fastness	Light fastness
1.	DG	91	84	3-4	3-4	4-5
2.	DA AG	93	87	4-5	4	4-5
3.	DS SG	96	91	5	4-5	5

5.2.6 Morphology of copper coated dyed cotton fabrics

Scanning electron microscopy (SEM) was utilized to analyze the morphological changes of cuprous oxide-coated dyed fabrics resulting from the application of different

reducing agents. The nanoscale images of cuprous oxide particles on the surface of dyed cotton fabric are depicted in Figure 40. Notable variations in size and surface morphology were observed among cuprous oxide particles reduced by different reducing agents.

Specifically, it was observed that dyed cuprous oxide particle-coated fabrics reduced by glucose exhibited larger particle sizes compared to those reduced by ascorbic acid and sodium hydrosulphite. While the comparatively smallest and even distribution of particles was observed in case of sodium hydrosulphite. The reason is that sodium hydrosulphite is the strongest and more compatible reducing agent for copper salts as compared to ascorbic acid and glucose [30][[31]. The weak reducing agent (glucose) provided the improper reduction of copper salt and produced the agglomerated structures, which in turn cover the less surface of fiber as shown in Figure 40c. The cuprous oxide particles reduced by sodium hydrosulphite and ascorbic acid covered the complete fiber surface (Figures 40b and 40a). Figure 8a showed the continuous and uniform distribution of particles on the surface of cotton. Furthermore, the deposition was found more uniform and denser with the increase in the concentration of copper salts.

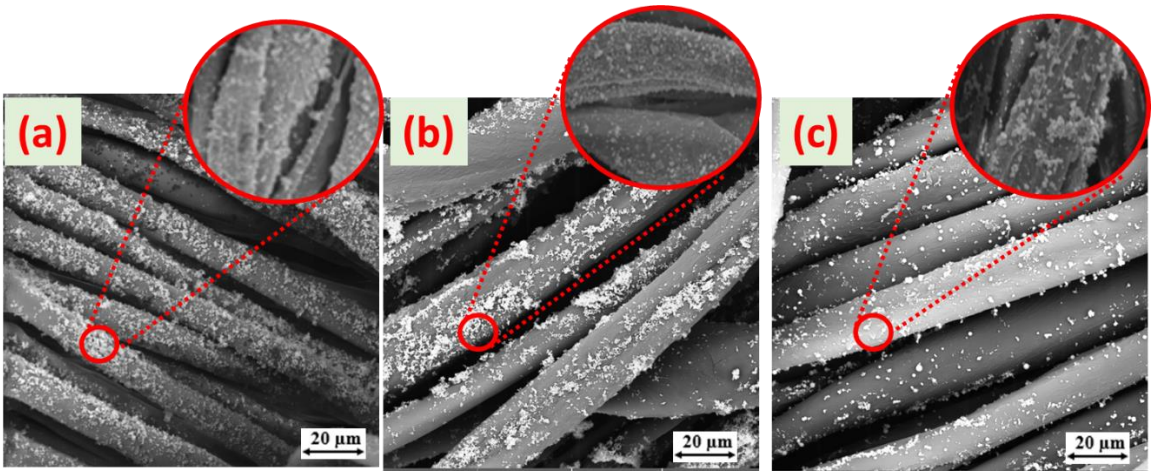


Figure 40: Surface morphology of dyed cotton fabrics coated with cuprous oxide particles (a) DS, (b) DA, (c) DG at 100x magnification and their close view at a magnification of 5kx.

5.2.*7* XRD analysis

The XRD analysis was conducted to determine the phase composition of the deposition of cuprous oxide particles. Figure 41 illustrates the XRD spectrum of the fabric sample in 2θ range of 10-80 degrees with a 0.02-degree shift. The precise identification of all the diffraction signals to the cuprous oxide structure reveals the phase purity of the produced cuprous oxide nanoparticles. The reflections that were represented by the diffraction peaks at 2θ of 29.6 o, 36.5 º, 42.4 º, 52.1 º, 61.5 º, and 73.7 º, respectively, were (1 1 0), (1 1 1), (2 0

0), (2 1 1), (2 2 0), and (3 1 1) [119] [50]. The sharpness of the signals validated the crystalline character of the cuprous oxide nanoparticles; however, the broadening of the signals supported the production of nanosized cuprous oxide particles. As such no characteristic peaks of impurities were detected, except the peak of the copper oxide phase at 2θ of 38^{o} [120][121].

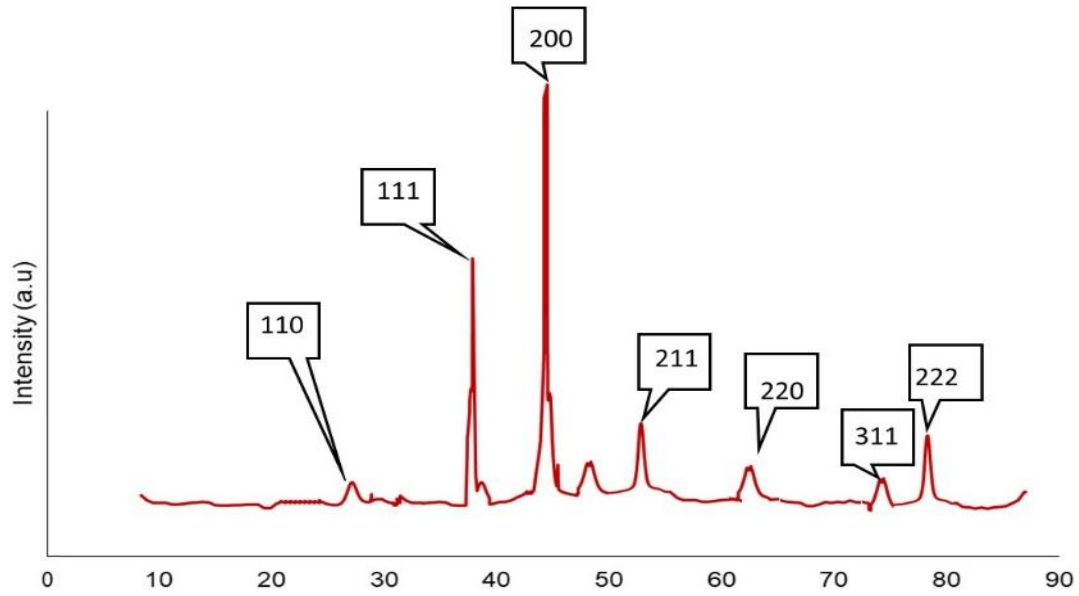


Figure 41: XRD patterns of cuprous oxide particles.

5.2.8 Antibacterial activity of the cuprous oxide nanoparticle coated fabrics

The antibacterial activity of copper-treated undyed and dyed fabric samples was tested both qualitatively and quantitatively using standard testing protocols.

5.2.8.1 Zone of inhibition test (qualitative measurements)

The AATCC-147 (disc-diffusion method) standard testing protocol was followed for the qualitative assessment of all treated samples. The antibacterial efficacy of all samples was evaluated against both Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria. The test was carried out three times and the mean value calculated for each sample is presented in Table 12. The obvious zones of inhibition around each fabric sample after 24 hours of incubation at 37 °C in the dark are depicted in **Figures 42 and 43**. All samples (undyed and dyed copper-coated fabrics) have shown significant zone of inhibition (ZOI) against both test microbes. However, it was observed that ZOI for dyed copper-coated samples is higher than for undyed copper-coated fabrics. The higher values of ZOI for dyed copper-coated fabrics revealed that the application of antibacterial dye on copper-coated fabric has not masked the antibacterial effect of copper particles, rather antibacterial effectiveness has increased after the dyeing of copper-treated fabrics.

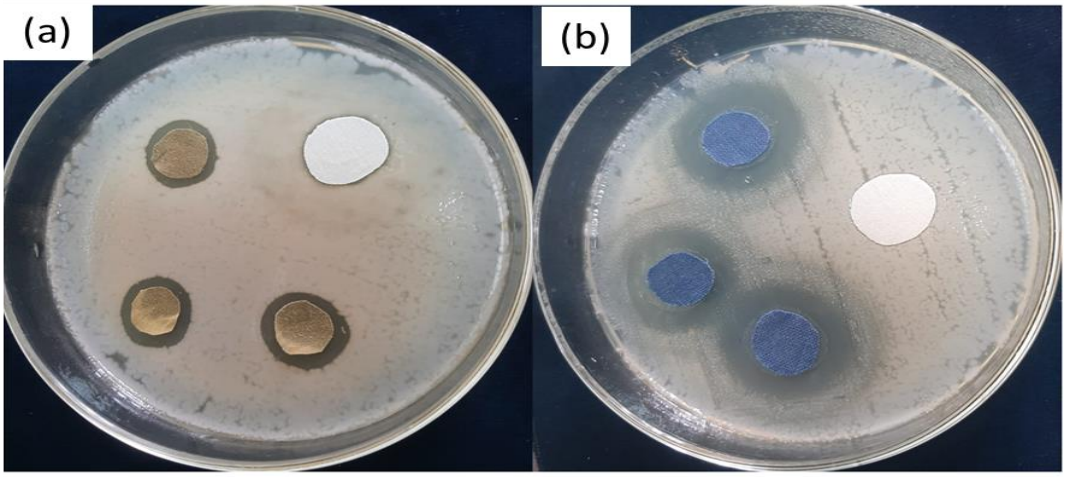


Figure 42: ZOI around (a) Copper-coated fabrics and (b) copper-coated dyed fabrics against *E-coli.*

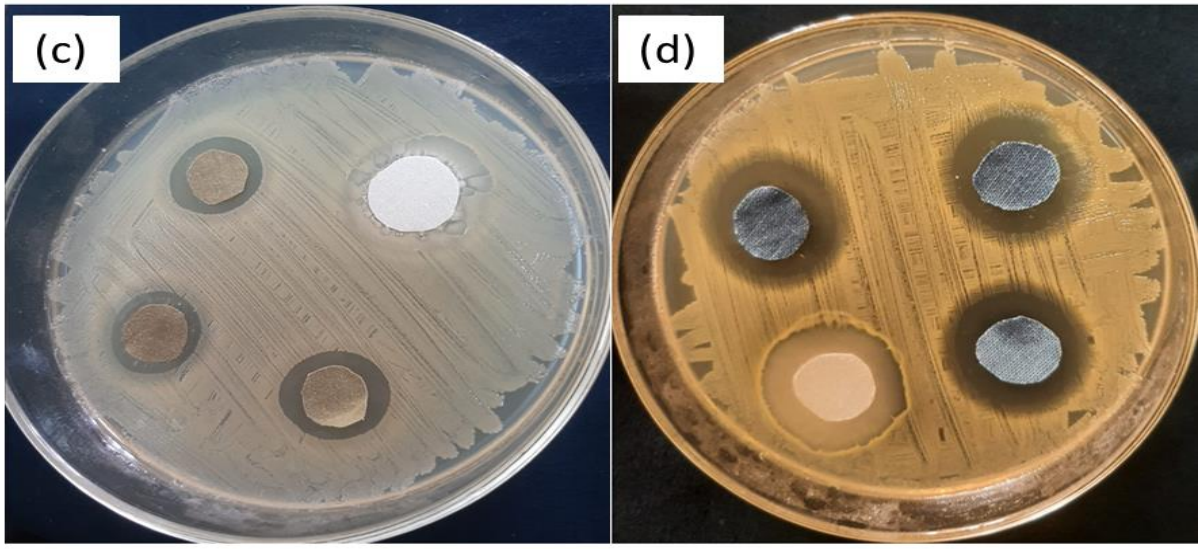


Figure 43: ZOI around (c) Copper-coated fabrics and (d) copper-coated dyed fabrics against *S-aureus.*

Table 11: The values of the zone of inhibitions against *S. aureus* and *E. coli.*

Sr.#	Sample Code	ZOI (mm)	
		S. aureus	*E. coli*
1.	UT	0	0
2.	G	5	2
3.	DG	7	3
4.	A	4	3
5.	DA	7	3
6.	S	5	3
7.	DS	8	4

5.2.8.2 Reduction factor (quantitative test)

ISO-20743 standard testing protocol was followed for the quantitative evaluation of the antibacterial efficacy of all developed samples against Gram-positive *(E. coli*) and Gram-negative (*S. aureus*) bacterial strains. The number of inoculated and survived bacterial colonies was taken and percentage reduction was calculated (Table 12). It was observed that all tested samples exhibited excellent antibacterial potential towards both test microbes i.e., >85% inhibition in bacterial growth. In case of samples G, A, and S, maximum antibacterial action was observed for sample S. It was also observed that the antibacterial activity of all samples increased significantly after the application of modified antibacterial dye on treated fabrics. The activity was increased from 87%, 90%, and 98%, to 99% for samples DG, DA, and DS, respectively against *E. coli*. The same increasing trend was observed for all 3 samples against *S. aureus*. The increase in antibacterial activity after the application of dye could be ascribed to the presence of a strong antibacterial agent i.e., triclosan which is reported to have an excellent antibacterial effect on a broad range of bacterial species. No antibacterial activity was observed for untreated cotton fabric (UT) which further confirmed that antibacterial activity in treated samples was due to the application of nanoparticles and antibacterial dye.

Table 12: The percentage reduction of developed samples against *S. aureus* and *E. coli.*

Sr.#	Reducing agent	Application of dye	Sample code	E. coli	S. aureus
1.	Untreated cotton	No	UT	0 %	0 %
2.	Glucose	No	G	87 %	91 %
3.	Glucose	Yes	DG	99.99 %	97.99 %
4.	Ascorbic acid	No	A	90.99%	95.99%
5.	Ascorbic acid	Yes	DA	99.9%	99.99%
6.	Sodium hydrosulphite	No	S	98.99%	99.99%
7.	Sodium hydrosulphite	Yes	DS	99.99%	99.99%

For more clarification, the reduction in colony-forming units (CFU/ml) of survived bacterial colonies was calculated, and obtained results are presented in Figure 44. The untreated cotton fabric exhibited a substantial number of survived bacterial colonies and higher values CFUs/ml were obtained (7.34 for E. coli and 6.44 for the S. aureus bacterial strain). The results revealed that CFUs concentration was remarkably reduced for all treated

samples. The samples S and DS exhibited the highest reduction in survived bacterial colonies and the CFUs values reached 0 from 7.34 and 6.44 for E. coli and S. aureus, respectively.

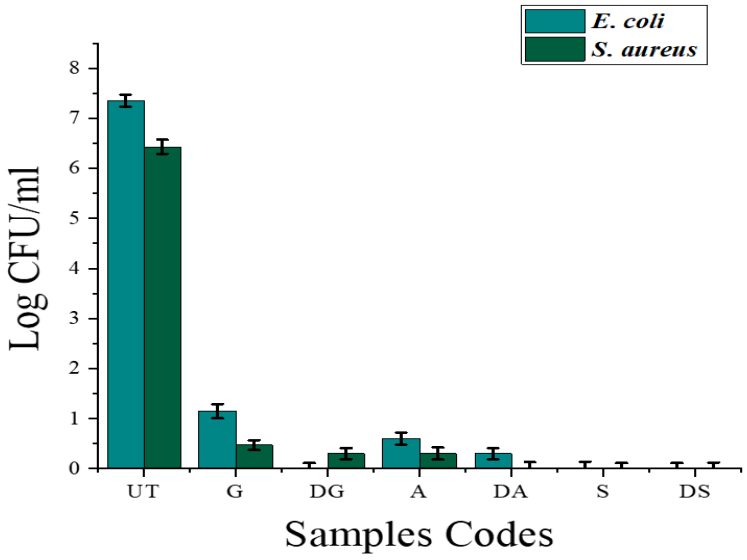


Figure 44: The reduction in CFUs of survived bacterial colonies for all developed samples

The validity of the obtained results was reaffirmed through the visual evidence depicted in Figures 45a and 46b, showcasing a clear comparison between untreated and treated samples against S. aureus and E. coli. The stark contrast between these images provides compelling insight into the efficacy of the antibacterial treatment.

In the case of untreated fabric samples, a discernible increase in the number of bacterial colonies is apparent, which strongly indicates the absence of any antibacterial action in the untreated (UT) sample. Conversely, for all treated samples, a remarkable reduction in the number of bacterial colonies is evident. This striking visual representation reinforces the fact that the treated samples exhibited a remarkable reduction of over 99% in bacterial growth, underlining the potent antibacterial effectiveness of the treatments.

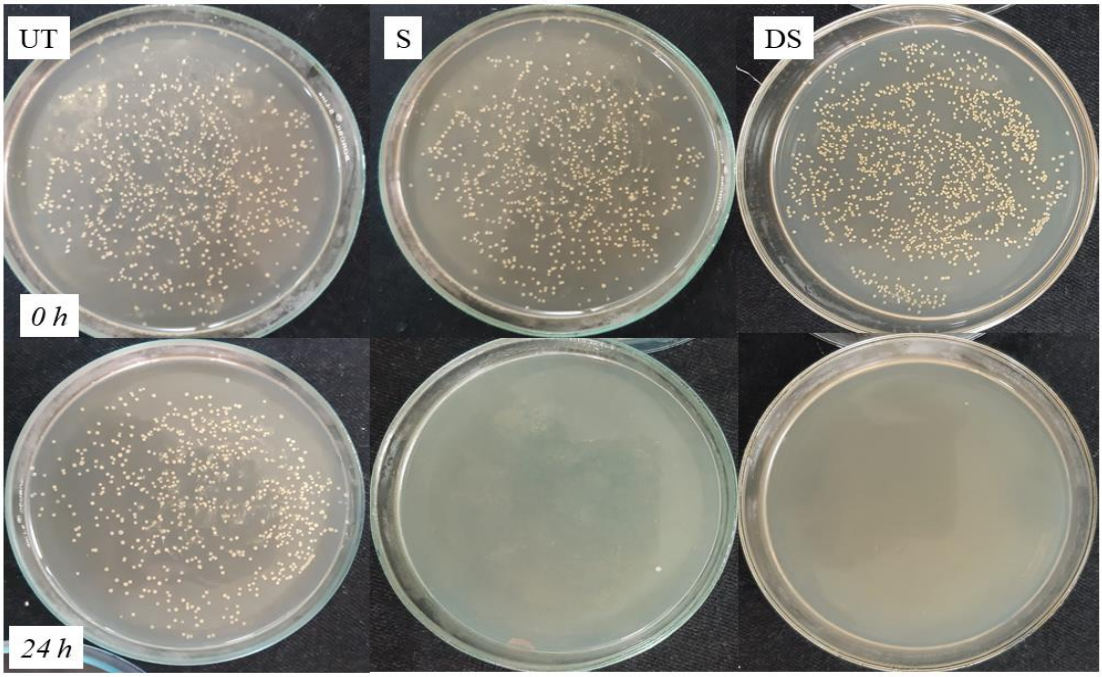


Figure 45:a: Images for the number of bacterial colonies inoculated (0 h) and survived (24 h) for sample UT, S, and DS against *S. aureus.*

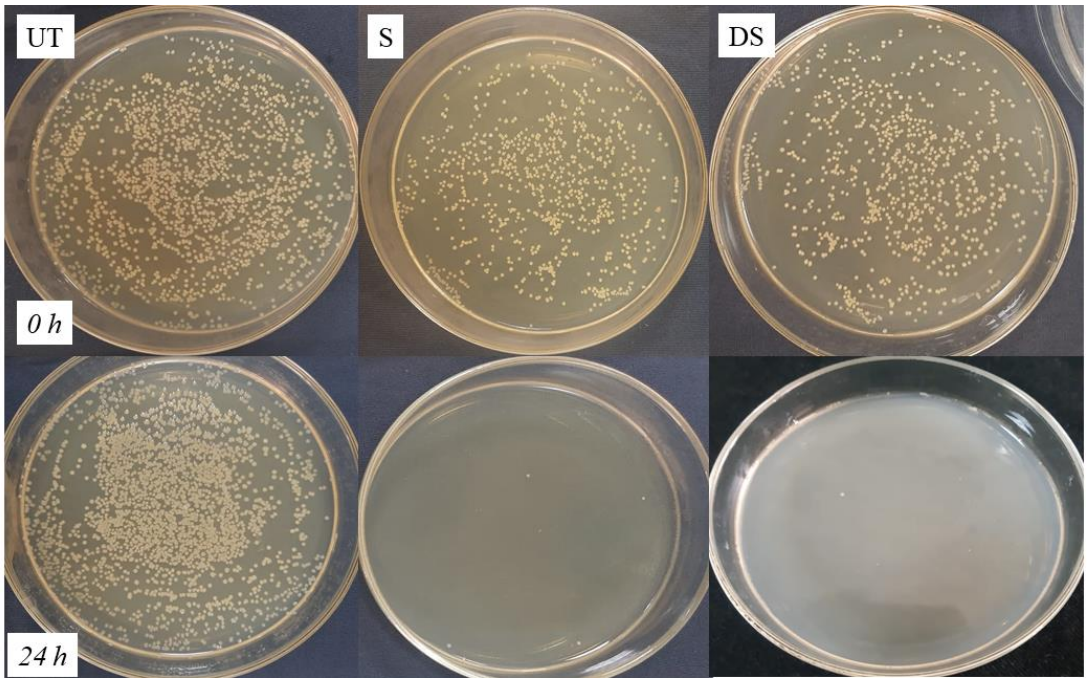


Figure 46:b: Images for the number of bacterial colonies inoculated (0 h) and survived (24 h) for sample UT, S, and DS against *E. coli.*

5.2.9 Antifungal Activity of Treated Samples

The quantitative assessment of antifungal activity against the A. Niger fungal species was conducted following the AATCC-100 standard method. The results, summarized in Table 13, showcase the percentage reduction in fungal growth achieved by all fabric samples,

both treated undyed and treated dyed. Impressively, all treated fabric samples, whether dyed or undyed, exhibited effective antifungal activity against the tested microbe. A noteworthy trend is observed, with the dyed samples demonstrating an increase in antifungal activity compared to their undyed counterparts. This enhancement could be attributed to the effective antifungal action of the incorporated dye itself. However, it's important to acknowledge that the antibacterial activity of the colored samples remains more pronounced than their antifungal counterpart. This disparity could be attributed to the nature of the incorporated triclosan, which has a higher efficacy against bacteria compared to fungi.

Among the undyed samples, sample S demonstrated the highest reduction in fungal spore growth, displaying a substantial antifungal activity of 89%. Similarly, among the dyed samples, sample DS exhibited exceptional antifungal action, attaining a maximum antifungal activity of 91%. The comparison against untreated fabric once again reiterates the ineffectiveness of untreated samples against the test microbe, thereby confirming that the observed antifungal activity in treated samples stems from the integration of nanoparticles and the modified antimicrobial dye.

Table 13: Reduction percentage of antifungal activity.

Sr.#	Reducing agent	Application of dye	Sample code	*A. Niger*
1.	Untreated cotton	No	UT	0 %
2.	Glucose	No	G	75%
3.	Glucose	Yes	DG	79 %
4.	Ascorbic acid	No	A	83%
5.	Ascorbic acid	Yes	DA	85%
6.	Sodium hydrosulphite	No	S	89%
7.	Sodium hydrosulphite	Yes	DS	91%

5.2.10 Antiviral effectiveness

Behrens and Karber's method were used for the evaluation of virus titers reduction from the initial viral titer of infectivity (10^8) against Corona Virus. Figure 47 showed the virus infectivity titer log at contact time (0 h and 60 mins). Figure 47 (a) showed the infectivity titer change of coronavirus for all tested samples. It was noticed that antiviral activity was increased for all dyed samples which indicated that dye has significant antiviral activity. However, the antibacterial action of dyed samples was more pronounced as compared to antiviral activity. This could be explained by the fact that the antibacterial action of triclosan is stronger than antiviral and antifungal action. The observed trend supported the

obtained results of the antibacterial activities of these samples. The viral infectivity titer was decreased significantly for all treated samples. However, the maximum reduction was exhibited by the sample S (among all undyed samples) and DS (among all dyed samples) which showed 79% and 83% antiviral action, respectively. The untreated fabric remained ineffective against the virus which further confirmed that antiviral action in all treated samples was due to the application of nanoparticles and modified antimicrobial dye. The antiviral action shown by the fabrics treated with copper nanoparticles and dyed fabric samples could be due to the binding of metallic NPs and the non-polar part (benzene ring) of the triclosan with glycoproteins at the viral surface working as an inhibitory action for viruses. The reduction percentages of all tested samples are given in Table 14.

Table 14: Reduction percentage of antifungal activity.

Sr.#	Reducing agent	Application of dye	Sample code	Corona Virus
1.	Untreated cotton	No	UT	0 %
2.	Glucose	No	G	70 %
3.	Glucose	Yes	DG	72 %
4.	Ascorbic acid	No	A	75 %
5.	Ascorbic acid	Yes	DA	75 %
6.	Sodium hydrosulphite	No	S	79 %
7.	Sodium hydrosulphite	Yes	DS	83 %

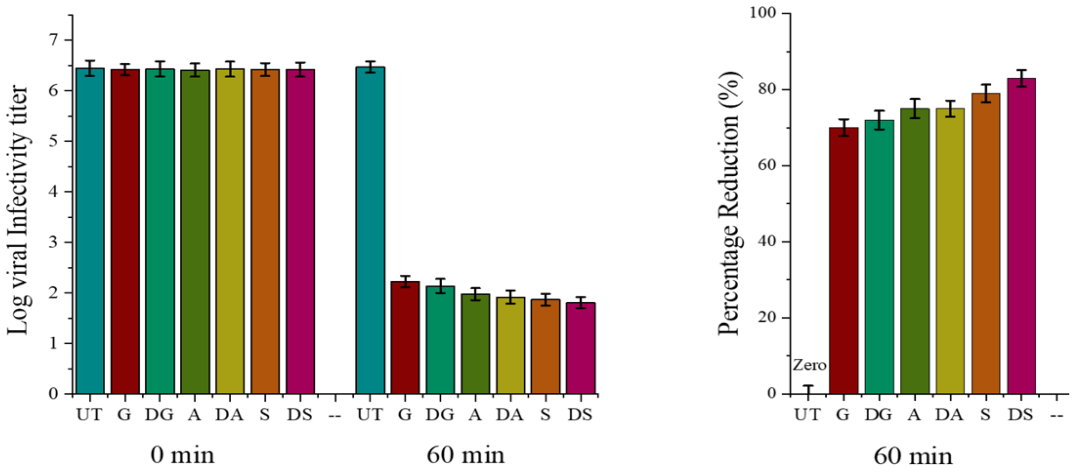


Figure 47: Reduction in viral infectivity titer (a) and percentage reduction (b) calculated from viral infectivity at a contact time of 0 and 60 min.

5.2.11 Durability of cuprous oxide coated fabrics

Copper nanoparticles (Cu-NPs) were previously noted to be attached to the fabric surface through a combination of physical and chemical linkages. The introduced Cu-NPs further aggregated the microfibers, effectively forming hygienic antibacterial networks by filling the gaps and voids between them. This property of absorption and adhesion was further underscored by the durability of antibacterial activity, even after repeated washing. To validate this behavior of absorption and adhesion, fabric samples coated with Cu-NPs were subjected to various tests. These included soaking, squeezing, and twisting the samples in water. Notably, the Cu-NP-coated samples exhibited favorable washing characteristics, with no observable peeling from the fabric surface or precipitation in the water. Subsequently, an adhesion test was performed using transparent tape. The tape retained its transparency, indicating the absence of detectable particles on its surface. This outcome serves to confirm the robust interactions and excellent mechanical adherence between Cu-NPs, dye molecules, and the fabric itself.

The durability of the antibacterial activity was assessed by evaluating the antibacterial properties of all treated fabric samples after undergoing washing cycles. Compliance with the ISO 105-C01 standard washing test protocol was followed. Table 15 provides a comprehensive overview of the antibacterial values for all tested samples, both before and after washing. The results clearly indicate that the inhibition zone values underwent negligible changes even after multiple washing cycles. Furthermore, this pattern was substantiated by SEM analysis carried out on the washed samples A1, S1, and G1, as depicted in Figure 20. The retention of nanoparticles on the fabric surface post-washing reaffirmed their strong adherence to the fibers and interstices. This evidence serves to emphasize the enduring nature of the antibacterial functionality of the treated fabrics, even after undergoing washing procedures.

Table 15: Antimicrobial properties of Cu_2O coated fabrics after washing.

Sr.#	**Sample Code**	**ZOI (mm)**		**ZOI (mm)**	
		Unwashed samples		Washed samples	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
1.	G	5	2	5	2
2.	DG	7	3	6	3
3.	A	4	3	4	2
4.	DA	7	3	7	3
5.	S	5	3	5	2
6.	DS	8	4	7	4

6. CONCLUSIONS

The cuprous nanoparticles have already been studied extensively due to their potential technological applications in medical fields. The main aim of the thesis was to investigate preparation properties and selected applications of bioactive textiles having antimicrobial ability, antiviral, antifungal, and durability. The first part of thesis was the formation of Cu_2O particles by the Fehling solution method and the effect of three different reducing agents was analyzed to prepare the Cu_2O particles. A total of nine hygienic multifunctional textile samples were developed using nanoparticle of cuprous oxide particles.

In the second step, the deposition of cuprous oxide particles was done on woven cotton fabric, and studied their antipathogenic (antibacterial, antifungal, and antiviral) properties. Surface morphology and the existence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. The antibacterial activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements. The strongest antibacterial effect was found for the fabrics coated with cuprous oxide particles reduced with sodium hydrosulphite at 1g/L.

Furthermore, the developed fabrics were analysed for comfort properties regarding air permeability and stiffness. The particles were so fine, ensuring that they did not obstruct the pores of the fabric structure. Hence, ensured improvement in stiffness and air permeability. In the end, the durability of deposition was confirmed by measuring the antibacterial properties and SEM analysis after washing. The retention of particles over the surface of the fabric reinforced the fact that particles are firmly attached to the fibers and interspaces. Developed process very easy, less in cost, and provide odorless work wear. The third study was to dye the already cuprous oxide-coated fabrics with antibacterial dye. The dyeing of the coated textiles has been performed to overcome discoloration and staining but their antibacterial effectiveness was compromised. Thus, the development of highly effective antimicrobial textiles with improved aesthetics was challenging.

In the second part, a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with an excellent aesthetic appearance was proposed. At first, fabrics were sensitized with citric acid. Then, the Fehling solution method was followed for the synthesis of Cu_2O nanoparticles. The synthesized nanoparticles were then applied to cotton fabric. Then, a reactive dye was selected and functionalized with an antibacterial agent. Subsequently, the cuprous oxide particles coated fabric were subjected to exhaust dyeing through the solution of functional bioactive dye. A total of 6 hygienic multifunctional textile samples (3 dyed and 3 undyed) were developed using nanoparticles of cuprous oxide

particles along with varying the concentrations of three different reducing agents. The surface morphology and existence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. After that, the Reactive blue 4 dye was functionalized with triclosan to impart antibacterial activity to the dye. FTIR and 13C-NMR results confirmed the successful modification of dye with an antibacterial agent. The modified dye was applied to copper-treated cotton fabrics through the exhaust dyeing method. The modified dye exhibited excellent fixation, exhaustion, and dye levelness on copper-treated fabric. The antibacterial activity of copper-treated fabrics was also increased after the application of dye. The antibacterial activity of cuprous oxide-coated dyed and undyed fabrics was tested against qualitative and quantitative measurements. It was observed that ZOI for dyed copper-coated samples is higher than for undyed copper-coated fabrics. The strongest antibacterial effect was found for the dyed fabric sample DS (sodium hydrosulphite). In case of quantitative analysis, the samples S and DS exhibited the highest reduction in survived bacterial colonies and the CFUs values reached 0 from 7.34 and 6.44 for E. coli and S. aureus, respectively. However, the antibacterial action of colored samples was more prominent in comparison to antifungal activity. This might be accounted to the fact that the antibacterial action of triclosan is higher than its antifungal and antiviral action. Furthermore, the durability of deposition was confirmed by measuring the antibacterial properties and SEM analysis after washing. The retention of particles over the surface of the fabric (SEM images) reinforced the fact that particles are firmly attached to the fibers and interspaces. Moreover, the change in antimicrobial activities of all treated fabric samples after repeated laundry cycles was insignificant which further confirmed the durability of particles on fabric. The developed process is very easy, less in cost, and provides odorless work wear. The successful application of cuprous oxide-coated fabrics explained their potential applications in the field of medical textiles to develop antibacterial surgical drapes, pants, socks, panels, bed sheets, surgical gowns, curtains, panel covers, wallpapers/sheets coverage, shoe mats, outlet covers, seat chair covers, Table covers, patient and doctors' socks, etc.

7. REFERENCES

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Organization: KellyDeli GmbH, Düsseldorf Germany **Designation:** Regional Manager
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Organization: Khawaja Sports Bornheim, Germany **Designation:** Textile sourcing and buyer
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List of Publications

1. **Muhammad Shahid**, Azam Ali, Jakub Wiener, Jiri Militky, Development of antimicrobial multifunctional Textiles to avoid from Hospital-Acquired Infections' Fibre and polymers, 2021; 22(11): 3055–3067
2. **Muhammad Shahid**, Azam Ali, Jakub Wiener, Jiri Militky, Copper-Treated Environmentally Friendly Antipathogenic Cotton Fabric with Modified Reactive Blue 4 Dye to Improve Its Antibacterial and Aesthetic Properties' Coatings, 2022; 13; 33 https://doi.org/10.3390/coatings13010133
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6. Azeem, M., Javed, A., Morikawa, H., Noman, M. T., Khan, M. Q., **Muhammad Shahid**., & Wiener, J. (2019). Hydrophilization of Polyester Textiles by Nonthermal Plasma. Autex Res. J, 142-149. 5.
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8. Azam Ali., **Muhammad Shahid**, M. and Jiri Militky., (2020). Copper based viral inhibition. 13th Textile Bioengineering and Informatics Symposium (TBIS). Manchester UK
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10. Silver-plated stretchable elastomeric electrodes for electrotherapy applications, Azam Ali, **Muhammad Shahid**, Nageena Zahid, Muhammad Shahzad Anjam, Tereza Subrova, Jakub Wiener, Jiri Militky, Blanka Tomkova, *fiber and polymers*, Accepted