Assessment of Antimicrobial Property of Naturally Coloured Cotton in Relation to Conventional White Cotton

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Abstract: ***Today's consumer is looking for both physical and emotional well-being. In recent years, enhancement of performance properties and imparting special properties to fabrics has become essential. One such smart technology for textiles is introduction of anti-microbial finishes which impart feeling of freshness and cleanliness to wearer. The micro-organisms that grow and thrive in warm, moist recesses of our clothing cause foul smell and morbidity. To make the garments suitable for intimate apparel and children's clothing, it is important to make them antimicrobial by applying dyes or selected finishes. Some common natural dyes have been found to exhibit antimicrobial property due to presence of large amounts of tannins. Tannins have the property to bind the microbial proteins, thus inhibiting their growth. As naturally coloured brown cotton has tannin derivatives and heavy metal ions as an integral part of its structure, it was considered imperative to explore and compare the antimicrobial property of coloured cotton with conventional white cotton. The present study was an endeavour in this direction where effort was made to explore the inherent antimicrobial property of naturally coloured cotton. The antimicrobial property of conventional white and naturally coloured cotton was assessed through Optical density and Standard Plate Count Test. The analysis of variance (ANOVA) highlighted that naturally coloured cotton significantly resisted the growth of microbes S. aureus, B. subtilis, E. coli and C. albicans.Amongst the coloured cottons, brown sample gave higher resistance due to the presence of catechin and other derivatives. The conidia and hyphae of fungus was hydrolysed due to catechin attack on the cell membrane. It also resisted bacterial growth by damaging the bacterial membrane. Gram positive bacteria exhibited better resistance because of the bactericidal effect of tannins present in naturally coloured cottons.***

Keywords: cotton, naturally coloured cotton, antimicrobial, S.aureus, E.coli, C. albicans, B. subtilis, catechin, fungus, tannins, performance properties, bacteria, fungus

I. INTRODUCTION

In the recent years, concern for environment has taken strong roots in the minds of people. Materials and products that are injurious to the ecosystem and human health are being increasingly discouraged. Environment consciousness and value addition are two important factors affecting the selection criteria of the consumers with high purchasing power.

The consumerhas become increasingly aware of hygienic life-style and want an environment and products which are environment friendly, health protecting and which prevent diseases. In this context, there is revival of interest in organic cotton and **naturally coloured cotton** without recourse to dyeing using harmful and polluting chemicals.The major hindrance that comes in way of achieving all of this is microorganisms (Gupta and Bhaumik, 2007). The microbes require only water, carbon source, nitrogen and some inorganic salts which are present in the natural environment.Many common natural dyes have been found to exhibit this property due to presence of large amounts of tannins (Gupta *et al*, 2005). Studies (Gupta and Laha, 2007; Gupta and Bhaumik, 2007) have also indicated that colour pigment and heavy metals/inorganic metal salts (mordants) also impart antimicrobial property to textiles.
As naturally coloured brown cotton has tannin derivatives as an integral part of its structure, it was considered imperative to explore and compare the antimicrobial property of naturally coloured cotton with conventional white cotton.

1.1 Microbes and their Effect on Human Health

Different types of microbes are grouped or classified as viruses, bacteria, algae, fungi and protozoa. Although, there are many diseases caused by these different microorganisms, but the major skin diseases in infants and adults are primarily caused by some species of bacteria and fungi.

1.1.1 Bacteria: Bacteria are self-sustaining astheydonot require any externalsource to multiply. Unlike common belief, all bacteria are not harmful. Only less than1% of bacteria are harmful and cause diseases. Amongst all age-groups, newborns are particularly susceptible to cutaneous and eye infections caused by bacteria like *S. aureus*, Coagulase- negative *staphylococci*, *P. aeruginosa* and *E. coli* (Prescot *et al*, 2003).

Classification of Bacteria:

Based on their response to gaseous oxygen

Aerobic bacteria-They require oxygen to thrive, grow and exist.

Anaerobic bacteria- These bacteria can exist only in absence of oxygen.

Facultative Bacteria- They can survive in presence and also absence of oxygen. They though prefer growing in presence of oxygen.

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Based on mode by which they obtain energy

Heterotrophs-They thrive on the energy that they obtain by breaking down the complex organic compounds.

Autotrophs- They make their own food by fixingcarbon dioxide. This process is facilitated by oxidation of elements like nitrogen and sulphur and by light energy. (Goyal, 2005).

Based on staining with the Gram stain

Bacteria have an outer rigid cell wall which protects them from osmoticlysis. Just inside the cell wall, is the cell membrane where oxidative phosphorylation occurs.

Gram positive- Gram-positive bacteria (e.g. .*Staphylococcus aureus*, *Bacillus subtillis*) are encased in a plasma membrane covered with a thick wall of peptidoglycan. Gram positive bacteria have no periplasmic space as is the case with Gram –ve bacteria. Gram +ve bacteria secrete exo-enzymes and they have extracellular digestion.

Gram negative- Gram negative bacteria (e.g. *E. coli*) have three layer casing.

Source: users.rcn.com/jkimball.ma.ultranet

FIGURE1.1: CELL STRUCTURE OF GRAM-POSITIVE AND GRAM-NEGATIVE

This outermost membrane contains lipopolysaccharide (LPS) and is the major permeability barrier. The periplasmic space stores degradative enzymes in gram –ve bacteria. This space is resent between the inner and outer membranes of Gram –ve bacteria. (Fox, *pathmicro.med.sc.edu/book/intro-sta.htm*). Figure 1.1 shows the difference between the cell structure of Gram positive and Gram negative bacteria.

1.1.2 Fungus: Fungi include mould, mildews and yeast. These are microscopic plant organisms but are not capable of synthesizing their own food. As compared to bacteria, fungi are infectious. These single cell organisms reproduce by budding. (Figure1.2 and 1.3). An example of yeast is the Candida species.

FIGURE 1.2: MICROSCOPIC FILM SHOWING

Source: *Fox, pathmicro.med.sc.edu/book/intro-sta.htm*

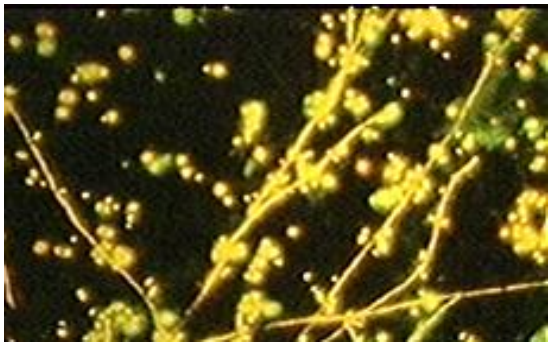


FIGURE 1.3: *CANDIDA ALBICANS* - YEAST AND HYPHAEA FLUORESCENT STAIN OF CANDIDA

1.2 Textiles: An Ideal Substrate for Microbes

Textiles mostly become an ideal substrate for bacterial and fungal cross infection or transmission of diseases. The transmission can occur through random contact or laundry operation (Saravanan, 2005). Both pathogenic and odour causing bacteria, interact an affect textilefibres in several phases. It may begin with initial adherence followed by growth and finally damage to the fibres and dissemination from them. The adherence of bacteria to fabrics is dependent upon

- Type of bacteria
- Physio-chemical properties of the fabric substrate
- Substrate and bacterial cell-wall hydrophobicity

Bacteria adherence and retention depends on two factors namely time of contact between the fabric and the microbe and the surface character of the fabric. Generally, rougher is the surface, the more is the retention (Gupta and Bhaumik, 2007). Natural and synthetic fibres act as most susceptible substrates for the microbial growth but the mechanism in both cases is quite different. **Natural fibres** like cotton, wool, hemp, jute and flax are most prone to microbial attack. It is so because oftheir porous and hydrophilic structure which retains water readily and the microbial enzymes can then easily hydrolyse their polymer linkages of these natural fibres e.g. if 10^5 colonies in 1ml water are applied to approx. 0.5g of cotton, after a few hours, a logarithmic growth is observed and the population increases from 10^5 to 10^9 colonies.

Whereas in the case of **synthetic fibres**, the rate of microbial growth is slower. The reason for this behavior is thatthey are hydrophobic in nature and their polymer backbone does not absorb and retain water well. But syntheticfibresdo retain stale perspiration in the interstices, wherein the microbes multiply at a very fast rate e.g. foot infection is more prevalent and acute for synthetic fibre socks as compared to natural fibre socks. Textiles are not only an ideal substrate and breeding ground but also plays an active role in propagating the microbes. Apart from adhering to the textile substrate and acting as active agents of cross infection; microbes also have detrimental effect on the fabric itself. Microbial growth in textiles increases with increasing moisture (due to body fluids), repeated launderings and neutral pH (7-8). Discoloration and loss of textile's functional properties such as loss of elasticity and textile strength generally occur due to the microbial attack on the additives like processing and finishing chemicals which are applied to the textiles. Microbes such as *Aspergillus niger* (Black mould) causes discoloration in textiles (Chaudhari, 2003). Further pigment production which can cause coloured stains on fabrics is a result of exposure to light.

1.3 Harmful Effects of Microbes on Human Health:

The human skin is possibly the most important barrier preventing microorganisms from entering our body. The human skin though a barrier but is an ideal breeding ground for bacteria because the metabolic by-products of body fluids such as acidic and basic perspiration and urine remain on skin (Kut*et al*, 2005). Bacteria and fungi are nearly always present on the human body. Even with clean skin has a typical population of between 100-1000 microbes/cm^2. At these levels, microbes pose neither a health orodour problem. In ideal conditions microbes can multiply rapidly and in just eight hours one bacterium can become 1.6 million. At this level number of bacteria, fungi and yeast can cause problems with odour and other possible infections (Goyal, 2005).

Body odour: Bacteria such as *S. aureus, S. epidermis* and *Corynebacterium* are found in the human skin and cause body odour. Body odour is the smell of bacteria excrement which consists of foul-smelling substances like carboxylic acid, aldehydes and amines (Roy Choudhury, 2008). The characteristic body odour is thought to be from 3-methyl-2-hexanoic acid produced by species of bacteria such as *Staphylococcus epidermidis*. In babies' diapers, the pungent odour is due to breakdown of urea into ammonia, by gram negative bacteria, *P. vulgaris* which secretes urease enzyme (Shah and Khanna, 2006).

Health problems: Most fungi and bacteria grow at ambient temperatures of 10-20^{o}C and some prefer the slightly warmer conditions of clothing or bedding that are in close proximity to skin. The moist skin and dark areas of groin, perineum and feet contain *S. aureus* and fungus *C. albicans* which produce skin infections. Clothing soiled by urine and faeces have been found to contribute to the growth of the *Brevibacteriumammoniagenes, E. coli* and *Proteus mirabilis*, which enhances diaper rash and this situation is associated with infections in infants.

Infectioncausing microbes in household and hospital environment and particularly in children are discussed below.

- *Staphylococcus aureus*

The pathogenic capacity of the Gram +ve bacteria, *S. aureus* (Figure 1.4) is due to the invasive property of the strain combined with the extracellular factors and toxins.

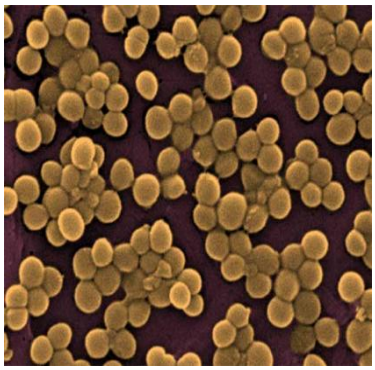


FIGURE 1.4: COLONIES OF *S. aureus*(magnified about 10,000x**)**

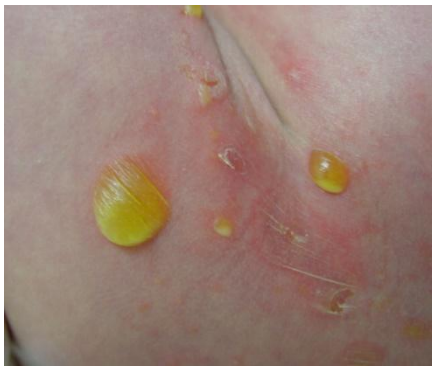


FIGURE 1.5: IMPETIGO CONTAGIOSA

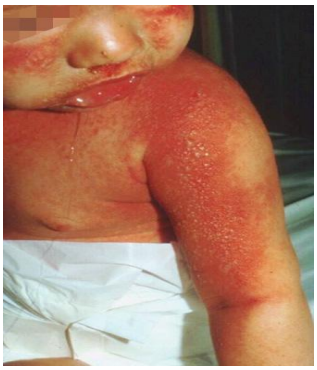


FIGURE 1.6: STAPHYLOCOCCAL SCALDED SKIN SYNDROME

Common diseases caused by *S. aureus*especially in case of children are Impetigo contagiosa. In this case, the newborn infants and children develop a superficial skin infection which is characterized by the presence of encrusted pustules (Figure 1.5). This disease is contagious and can spread through a nursery or school. Another disease commonly found in infants and children is Staphylococcal Scalded Skin Syndrome (SSSS). In SSSS, the epidermis peels off to reveal the red area underneath (Figure 1.6).

- *Escherichia coli*

*Escherichia coli*causes infections liketravellers'diarrhoea and children's diarrhoea. The *E. coli* bacterium is a large, unmoveable Gram-negative stick (Figure 1.7 and 1.8) first described by Escherich. It causes diaper rash or nappy rash in infants. *E. coli* is generally found in the gut and is also known to cause cholera-like diseases and bloody diarrhea.

FIGURE 1.7: A CLUSTER OF *E. COLI* BACTERIA

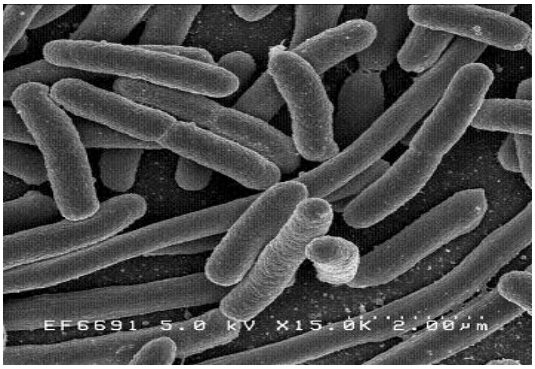


FIGURE 1.8: CELL STRUCTURE OF *E. COLI* (MAGNIFIED 10,000 TIMES)

Source: Fox, pathmicro.med.sc.edu/book/intro-sta.htmSource:images.google.co.in

- *Candida albicans*

The skin yeast infections are all commonly caused by *candida albicans* and *candida tropicalis.* Both *C. albicans* and *C. tropicalis* are most common fungal infection causing fungi for humans. Fungi increase the humidity of the skin and reduce fat content. These partly digested skin organs thus are converted into food particles for the mites or fungus. This can increase the skin diseases like asthma, allergies and eczema (Sampath, 2003). **Candidiasis** is the mycosis caused by the dimorphic fungus ***Candida albicans***. It produces a large variety of diseases in humans. Most infections involve the skin or mucous membranes because it is an aerobe and finds such surfaces very suitable for growth. Intertriginous candidiasis involves those areas of the body, usually opposed to skin surfaces that are warm and moist: axillae, groin, and skin folds. Napkin (Diaper rash) candidiasis is typically found in infants (Prescot *et al*, 2003). Figures 1.9 and 1.10 illustrate the effect of Candidiasis.

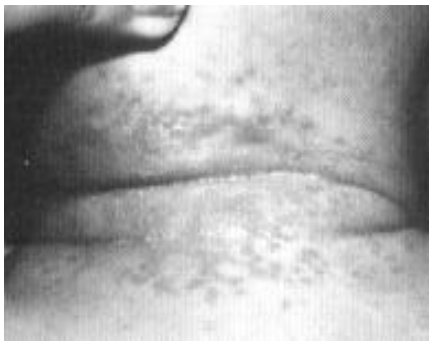


FIGURE 1.9: SKIN YEAST INFECTION ON A BABY WITH A HEAT TYPE RASH ON ITS NECK

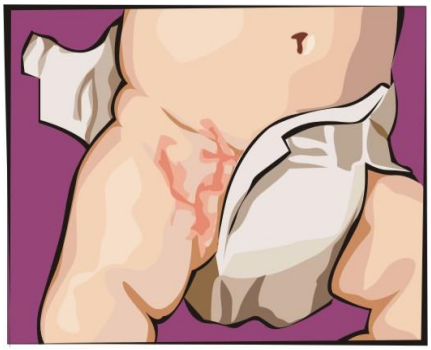


FIGURE 1.10: YEAST INFECTION IS THE WORST TYPE OF DIAPER RASH

II. METHODS AND MATERIALS

Naturally coloured cotton is one of the eco-friendly alternatives to conventional white cotton as it eliminates the chemical intensive processes of dyeing and finishing. Though naturally coloured cotton has existed in India since time immemorial, yet there is a need to compare properties of naturally coloured cotton with conventional white cotton.One such desired property is antimicrobial. Many common natural dyes have been found to exhibit this property because of the presence of large amounts of tannins in them (Gupta *et al*, 2005). As naturally coloured brown cotton has tannin derivatives as an integral part of its structure, it was considered imperative to explore and compare the antimicrobial property of naturally coloured cotton with conventional white cotton. Further, naturally coloured cotton has also been credited with high heavy metal ion content and it was postulated that this would also significantly affect the performance properties of these fibres.

The study included the experimental work and the experiments were conducted in laboratories of Northern India Textile Research Association (NITRA); Ghaziabad, Institute of Home Economics, New Delhi, Textile Technology Department, Indian Institute of Technology, New Delhi and Department of Anatomy, All India Institute of Medical Sciences, New Delhi.

Sample selection: Plain single jersey knitted samples of 24^s count from four cotton samples namely, naturally coloured brown (BD and BN), green (GN) and conventional white cotton (WM) were selected to assess the role of pigment on the performance properties of cotton. Two naturally brown coloured cotton samples from different regions (BD and BN from Dharwad and NITRA fields respectively) were selected to ascertain the role of heavy metals ions on the property under consideration.

2.1Assessment of Anti-Microbial Activity

The human skin is an ideal breeding ground for bacteria due to the presence of metabolic byproducts of body fluids like perspiration and urine (Kut*et al*, 2005). Bacteria like staphylococci are ubiquitous and all people have it on their skin and transient colonization is common with *Staphylococcus aureus* particularly in warm, moist skin folds. *Staphylococcus aureus* is one of the main reasons of skin infections (Murray *et al*, 1990). The yeast, *Candida albicans* is also a culprit in causing many skin diseases especially in infants.

In the present study, the behavior of naturally coloured cotton towards different microbes was studied and compared with conventional white cotton. The quantitative assessment of microbial activity was determined by Turbidity test method and by Standard Plate Count Method.

2.1.1Test Organisms or Cultures

The main skin-infection causing bacteria of each Gram-negative and Gram-positive strains and also a fungus were taken for performing the antimicrobial tests.

Gram-positive: *Staphylococcus aureus, Bacillus subtilis*
Gram-negative: *Escherichia coli*
Fungus: *Candida albicans*

2.1.2 LIQUID CULTURE MEDIUM

There are some essential requirements for microorganisms to grow like basic nutrients and certain physical factors. To successfully cultivate the microorganisms in laboratory it is important ad essential to satisfy the diverse nutritional needs of microbes. There are two major categories of media used for routine cultivation of microorganisms.

- Artificial Media- They are composed of a limited number of complex substances, plant and animal extracts, whose exact compositions are not known. They are capable of supporting the growth of most heterotrophs which cannot be cultivated in a medium consisting solely of inorganic compounds and must be supplied with organic nutrients.
- Chemically Defined Media- These are composed of known quantities of chemically pure, specific organic, and/or inorganic compounds. Their use requires knowledge of the organism's nutritional needs.

Preparation of Nutrient Broth (Artificial Medium) for Bacteria

The nutrient broth contains peptone and beef extract. Peptone is a semi-digested protein, primarily a nitrogen source. Beef extract, a beef derivative is a source of organic carbon, nitrogen, vitamins and inorganic salts. This basic artificial medium was prepared (Cappuccino and Sherman, 1992) by incorporating beef extract and peptone per 1000 ml of distilled water. All the ingredients were mixed and heated to boil to disperse ingredients. The pH was adjusted to 7 with 1N Sodium hydroxide solution. The prepared broth, 10ml each, was then dispensed in two test tubes, which were cotton plugged and sterilized at 120°C, 15psi for 15 minutes. After sterilization, with the help of a 4 mm inoculating loop, Gram positive and Gram negative bacteria were inoculated from mother culture into the freshly sterilized liquid nutrient broth and incubated at 37°C for 24 hours.

Preparation of Yeast Extract Broth for Fungus

The yeast extract broth is an example of an enriched medium. It is used for the cultivation of fastidious microorganisms i.e. organisms that have highly elaborate and specific nutritional needs. They do not grow or grow poorly on a basic artificial medium and require the addition of one or more growth-supporting substances such as additional plant or animal extracts, vitamins or blood. *Candida albicans*, a fungus requires this broth for its growth where yeast extract is added to the broth to provide Vitamin B and additional organic nitrogen and carbon compounds. This broth was prepared (Cappuccino and Sherman, 1992) by incorporating ingredients in 1000 ml of distilled water.All the ingredients were mixed properly. The pH was adjusted to 5.6 with 1N Hydrochloric acid solution. The prepared broth, 10ml each, was then dispensed in two test tubes, which were cotton plugged and sterilized at temperature 120°C and pressure 103KPa (15psi) for 15 minutes. After sterilization, with the help of a 4mm inoculating loop, fungus, *Candida albicans* was inoculated from mother culture into the freshly sterilized liquid nutrient broth and incubated at 37°C for 24 hours.

Inorganic Synthetic Broth (Chemically Defined Medium)

All organisms require a source of carbon for synthesis of the numerous compounds which comprise protoplasm. In order to study the role of cellulose alone as available nutrition material for bacteria, inorganic synthetic broth was also used which is devoid of any carbon source. The results were compared with the ones obtained with nutrient broth.(Cappuccino and Sherman, 1992)

2.1.3 SOLID CULTURE MEDIUM

Nutrient Agar from Himedia, code M001-500G was used to prepare the solid culture medium.

Preparation of Nutrient Agar (for Bacteria)

Nutrient Agar was added to 1000 ml of distilled water. It was heated and brought to a boil to dissolve the medium completely. The pH was adjusted to 7.4 $\pm$ 0.2 using 1N Sodium hydroxide solution. The solution was dispensed in flasks, cotton plugged and sterilized by autoclaving at 120°C, 103KPa (15 psi) pressure for 15 minutes.

Preparation of Nutrient Agar (for Fungus)

Bacteriological Agar was added to nutrient broth as prepared above, heated and brought to a boil. The pH was adjusted using 1N Sodium hydroxide solution. It was then dispensed in flasks; cotton plugged and sterilized by autoclaving at 120°C, 15 psi pressure for 15 minutes.

2.1.4 TESTS TO ASSESS MICROBIAL ACTIVITY OF COTTON SAMPLES

Quantitative tests were performed to assess the antimicrobial behavior of naturally coloured brown and green cotton in relation to conventional white cotton. The four cotton samples namely WM, GN, BD and BN were tested against all the selected test organisms. The quantitative assessment of microbial activity in each of the cotton samples were carried out by standard test methods set by American Association for Testing Chemicals and Colorist. Turbidity Test and Standard Plate Count Method (AATCC

100-2004) were used to ascertain the antimicrobial efficiency of samples. All tests were done in microbiology laboratory environment of about 24°C and 55% relative humidity and repeated three times. The results recorded in figures were an average of three readings for each parameter.

2.1.4.1 Assessment of antimicrobial behavior through Turbidity Test: Thedegreeof antimicrobial activity was recorded using a spectrophotometer which records the absorbance of the sample (Kumar and Krishnaveni, 2007). The absorbance values of four fabric samples with selected test organisms were measured and compared.

Principle: Greater the bacterial/fungal growth, higher is the turbidity. The optical density therefore is directly proportional to the number of bacteria in the medium (Kumar and Krishnaveni, 2007).

2.1.4.2 Assessment of antimicrobial behavior through StandardPlate Count TestMethod: The microbial activity of fabrics was tested using the AATCC 100-2004 test method by counting the number of colony forming units (CFU) (AATCC Technical Manual, 2009; Gupta and Laha, 2007). This test gives a quantitative assessment of the antibacterial property of the test specimen. The method is technically more difficult and time consuming where the textile specimen is inoculated with a bacterial suspension in aqueous nutrient and after incubating for 24hrs, the viable bacteria are counted (Goyal, 2005).

Principle: Test and control swatches are inoculated with the test organisms. After incubation, the bacteria are eluted from the swatches by shaking in known amounts of neutralizing solution. The number of bacteria present in the liquid is determined and the percentage reduction by the treated specimen is calculated (AATCC Technical Manual, 2009).

To compute the estimated number of bacteria on the surface that had been tested, the following formula was used (Roy Choudhury, 2008) which gives viable spores per gram:

$$B = N \times D$$

Where,

B = number of bacteria

N= number of colonies counted on plate

D=dilution factor

To draw comparison, reduction in the number of bacteria was calculated using the following equation:

$$\text{Reduction rate (\%)} = [(B-A)/B] \times 100$$

Where,

A = number of bacteria recovered from the inoculated test specimen swatches over the desired contact period

B = bacteria recovered from the inoculated test culture after desired contact period

(AATCC Technical Manual, 2009; Orhan *et al*, 2007)

2.1.5 Antimicrobial Activity of Conventional White, Green and Brown against Bacteria and Fungus

Brown samples, both from NITRA (GN) and Dharwad (BD) were tested for ascertaining the role of natural pigment and/or heavy metal ion content on the microbial activity of the fabric samples. The results obtained were subjected to statistical analysis carried out by SPSS statistics program (Version 15) to conclude if the difference in the microbial behavior of coloured cotton and white cotton was significant or not.

The significance of the results obtained was statistically understood from the variation analysis (ANOVA) with 95% confidence level. Analysis of Variance (ANOVA) was used as a statistical tool to find the ratio of two variances (i) between samples and (ii) within the samples. The purpose was to find out the influence of difference forces working on them. Analysis of Variance or '*F*-Test' technique developed by Sir Ronald Fisher in 1920's was developed to test for the significance of the difference among more than two sample means and to make inferences about whether such samples were drawn from the populations having the same mean (Gupta, 1996; Jhunjhunwala, 2008). ANOVA thus helps in analyzing the variation of data into components which may be attributed to various "sources" or "causes" of variation.

Tukey's HSD (Honestly Significant Difference) Tests were also performed to determine where significant differences existed. The test helps in preventing any kind of error while declaring the difference to be statistically significant (Miniu*met al*, 2001).

The above tests gave a comparative assessment of the antimicrobial property of the selected naturally coloured and white cotton samples. The resistance against selected bacteria and fungus was quantitatively ascertained and helped establish the relation between pigment, heavy metal ions and the microbial-resistant property of selected fabric samples.

III. RESULTS AND DISCUSSION

Cotton textiles are the most preferred textiles for infants because of its properties like soft hand and absorbency, but it has certain disadvantages too. Natural fibre like cotton is an ideal growth environment for bacteria, yeast and fungi. Essentials like nutrition, water and oxygen that are required for the growth of these organisms are easily satisfied in these textile materials.

It thus becomes imperative to have a product which has all the good properties of cotton but at the same time does not facilitate for microbial growth. In the present section the effect of natural pigment and high heavy metal ion content present in naturally coloured cotton was studied on the microbial activity on the fabrics. Four microbes- Gram positive, *Staphylococcus aureus, Bacillus subtilis;* Gram negative *Escherichia coli* andfungus *Candida albicans* were selected. The naturally coloured and white cotton samples were exposed to these select microbes to study the inherent antimicrobial property exhibited by varieties of naturally coloured cotton.

3.1Heavy Metal Analysis

The quantitative analysis of heavy metal ions was done using Atomic Absorption Spectrophotometer and the results are tabulated in Table 3.1. The amount of heavy metal ions was significantly higher in naturally coloured cotton samples than white cotton.

TABLE 3.1: HEAVY METAL ANALYSIS IN COTTON SAMPLES

Heavy metals	Values in mg/kg of fibre			
	Brown Sample		Green Sample	White Sample
	BN	BD	GN	WM
Lead	2.80	3.86	1.87	3.14
Cadmium	0.10	0.125	0.20	0.312
Chromium	Not Traceable	Not Traceable	Not Traceable	Not Traceable
Copper	1.00	5.062	1.30	1.25
Zinc	6.05	13.54	4.17	5.31
Nickel	0.65	0.20	1.40	0.30
Cobalt	0.55	0.625	0.85	0.313
Iron	10.6	17.86	11.3	5.86
TOTAL	**21.75**	**41.27**	**21.09**	**16.48**

Table 3.1 shows that the heavy metal chromium was not traceable in all the cotton samples. The other heavy metals like zinc, cobalt and iron were higher in coloured cotton samples than white cotton samples. Nickel was significantly higher and lead was lower in green coloured cotton samples. No specific relation was observed in case of heavy metal, cadmium. The amount of copper present in all samples was comparable barring sample BD, from Dharwad which exhibited exceptionally high amount of the metal (5.062mg/kg of fibre).

The overall results indicated maximum amount of heavy metals in brown naturally coloured cottons followed by green and white cotton samples. The amount of heavy metals present in the two green coloured cotton varieties were comparable but a lot of variation was observed in the three brown cotton samples. Of the naturally brown coloured cottons least amount of heavy metals were detected in naturally brown coloured sample BN from NITRA (21.75mg) followed by BD (41.272mg).

3.2 Assessment of Antimicrobial Behavior

Two approaches were used to quantify the amount of microbial growth

1. Measurement of optical density using spectrophotometer
2. Counting the Colony Forming Units (CFU)

3.2.1 Assessment through Optical Density

Quantitative assessment was done by taking the optical density readings of the inoculated and incubated samples at λ=600nm with the help of Spectrophotometer (Systronics-Visiscan 167). The tests were performed in triplicate and results were recorded as an average of three readings taken in each case.

The optical density is directly proportional to the number of bacteria in the mediumbecause greater the bacterial growth, higher is the turbidity. (Kumar and Krishnaveni, 2007).

TABLE 3.2: OPTICAL DENSITY ANALYSIS OF SCOURED COTTON SAMPLES

Sample	Optical Density reading (λ=600nm)						
	S. aureus		*B. subtilis*		*E. coli*		*C. albicans*
	Nutrient broth	Minimal medium	Nutrient broth	Minimal medium	Nutrient broth	Minimal medium	Yeast Extract broth
Control (microbe)	1.150	0.072	1.872	0.159	1.929	0.105	1.924
WM	1.208	0.254	1.960	0.294	1.945	0.285	2.079
BD	0.643	0.035	1.581	0.153	1.476	0.179	1.110
BN	0.714	0.087	1.602	0.177	1.532	0.198	1.380
GN	0.995	0.184	1.670	0.196	1.701	0.240	1.629

Cotton is an easy target for microbial attack because it is a hydrophilic fibre and can retain water easily and thus the microbial enzymes can readily hydrolyze their polymer linkages. Therefore cellulose present in the fibre promotes microbial growth. To explore the antimicrobial property of naturally coloured cottons, they were exposed to select microbes both in nutrient broth and in minimal medium.

The results tabulated in Table 3.2 show maximum turbidity implying maximum microbial growth in case of white cotton WM sample and less in naturally coloured brown and green scoured samples. To further establish this phenomenon, the microbe culture inoculated samples were also kept for 24hrs at 37°C in the minimal medium broth, minus the carbon source so that nourishment was provided from the cellulose present in the fibre alone. Similar trend was achieved as with nutrient broth and this was indicative of the fact that white cotton showed maximum microbial growth and naturally coloured cotton samples showed some inherent antimicrobial property.

The results also indicate that though the different cotton samples have similar cellulose composition they exhibited significantly different results (Figure 3.1). Brown and green coloured cotton show low optical density values, indicating antimicrobial property, than white cotton, probably due to the presence of high content of heavy metal ions like zinc and copper (Table 3.1) which act as germicides (Prescot *et al*, 2003).

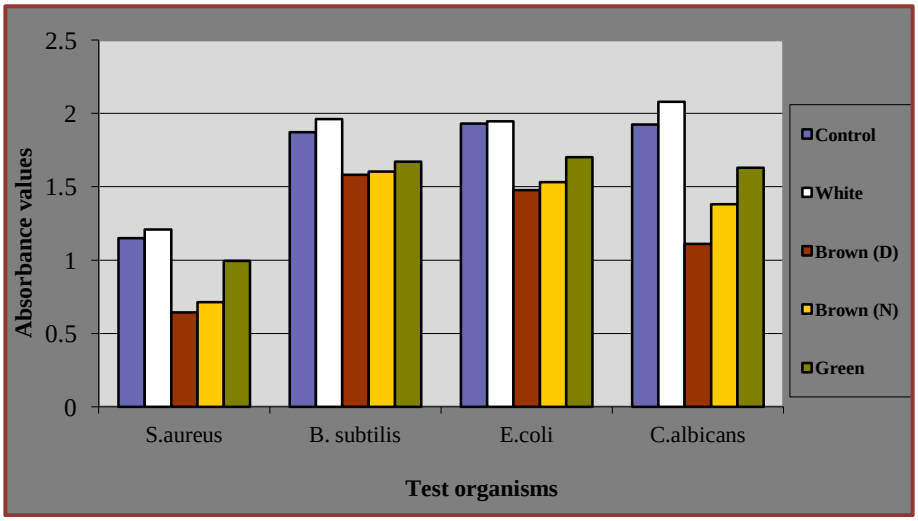


FIGURE 3.1: COMPARATIVE ANALYSIS OF ABSORBANCE VALUES OF COTTON SAMPLES

To establish the role of heavy metals against the selected microbes, two samples of brown cotton grown in different regions with varying heavy metal ion content were tested. Brown (BD) with heavy metal content of 41.272mg/kg of fibre exhibited lower optical density values or higher antimicrobial behavior as compared to brown fibre BN from NITRA fields which had heavy metal content of 21.75mg/kg of fibre (Table 3.1). This indicated that presence of heavy metal increases the antimicrobial property. Further, the difference between the heavy metal content of brown (BN) and green (GN) was marginal (0.66 mg/kg of fibre) but the results indicated significant difference probably due to combined effect of metal ions and tannins present in brown cotton samples.

When evaluating various microbes, absorbance values were found to be least with *S. aureus*, indicating highest antimicrobial property of naturally coloured cottons against this bacterium. In case of *B. subtilis* and *E. coli*, these values were higher and comparable for all cotton samples. Resistance against *S. aureus* is of particular importance as they are known to cause numerous skin infections (Prescot *et al*, 2003) and have the ability to remain attached on textile substrate after multiple detergent wash cycles. This phenomenon would be more critical for infant clothing where microbial growth in textiles increases with increasing moisture (due to body fluids), repeated launderings and neutral pH (ideal for microbial growth) (Chaudhari, 2003).

In case of Gram negative bacterium, *E. coli* the absorbance values were higher probably due to the presence of an additional layer; the outermost membrane that contains lipopolysaccharide (LPS) and acts as the major permeability barrier for any antimicrobial substance (heavy metal ions) to have adverse effect on the bacterium.

The absorbance values for fungus, *Candida albicans* were maximum with white WM cotton sample. According to Gupta and Bhaumik (2007), this is due to the presence of high concentration of microbial cells and hyphae in the structure of the fungus. These hyphae also directly damage the secondary wall of cotton and then start growing inside the lumen. Fungal hyphae are coarser (5µm) than the cotton pore (16Å). In some cases, it penetrates the lumen without breaking the outside surface.

The overall results indicated least microbial growth in brown naturally coloured cotton followed by green and white cotton samples. The method successfully enumerated the number of cells in a bacterial culture but the major disadvantage of the test method was that the total count includes dead as well as living cells. Therefore, to determine the viable cells present in the culture, the serial dilution-agar planting technique was also used to quantify the inherent antimicrobial property of naturally coloured cotton.

3.2.2Assessment of Antimicrobial Behavior throughStandardPlate Count TestMethod

The quantitative Standard Plate Count Test (AATCC 100-2004) methodwas used to corroborate the above findings by counting the number of viable colony forming units (CFU) formed in the selected cotton samples.

TABLE 3.2:ANTIMICROBIAL BEHAVIOR OF COTTON SAMPLES BY STANDARD PLATE COUNT TEST METHOD

Sample	**Colony Forming Units** (dilution 10^7)			
	S. aureus	***B. subtilis***	***E. coli***	***C. albicans***
Control (microbe)	267	TNTC*	TNTC	TNTC
WM	TNTC	TNTC	TNTC	TNTC

BD	135	143	252	108
BN	178	164	293	126
GN	221	182	TNTC	157

**Too Numerous To Count*

Table 3.2shows that white scoured cotton sample (WM) developed dense population of microbial growth with all the four microbes and the colonies were too dense to be counted accurately, hence recorded as TNTC. Both naturally coloured brown and green scoured samples showed remarkably less number of colonies with all test organisms as compared to control and white cotton sample. Naturally coloured cotton samples exhibited maximum inhibition against *C.albicans* followed by *S. aureus, B. subtilis* and *E. coli* (Plate 3.1). Naturally brown coloured cotton samples BN and BD gave least number of 108 and 126 colony forming units against fungus *C. albicans*. This was followed by a comparatively higher CFU (156) with green GN cotton sample. The CFU obtained with Gram-negative bacteria, *E. coli*was relatively higher or too numerous to count probably due to an additional outer membrane present in the structure of Gram negative bacteria which poses as an additional barrier for any antimicrobial agent (heavy metal ions) to act on the microbe.

Brown pigmented samples BD and BN showed least overall microbial growth, hence has better antimicrobial property than white and green pigmented cotton (Figure 3.2). This property could be attributed to the tannin precursor, catechin and other tannin derivatives present in the brown fibre as tannins help in imparting anti-microbial property (Gupta *et al*, 2005). Tannins are water soluble poly-phenols that are of two types- Tannic acid (hydrolysable) and the non-hydrolysable, catechin (Akiyama *et al*, 2001). Tannins act as antimicrobials through inhibition of extracellular microbial enzymes or direct action on microbial metabolism through inhibition of oxidative phosphorylation (Scarlbet, 1991).

Brown cotton gave least CFU with *C. albicans* as catechin attacked the cell membrane and caused lysis of the conidia and hyphe present in the fungus. In case of *S. aureus* and *E. coli*, catechins act upon and damage the bacterial membranes (Masatomo and Kazuko, 2004). Naturally brown coloured cotton exhibited better inhibition for Gram-positive (*S. aureus*) than Gram-negative (*E. coli*) bacteria because Gram-positive bacteria are more prone to the bactericidal effect of tannins than Gram-negative bacteria (Gupta and Laha, 2007; Tsutomu, 2003).

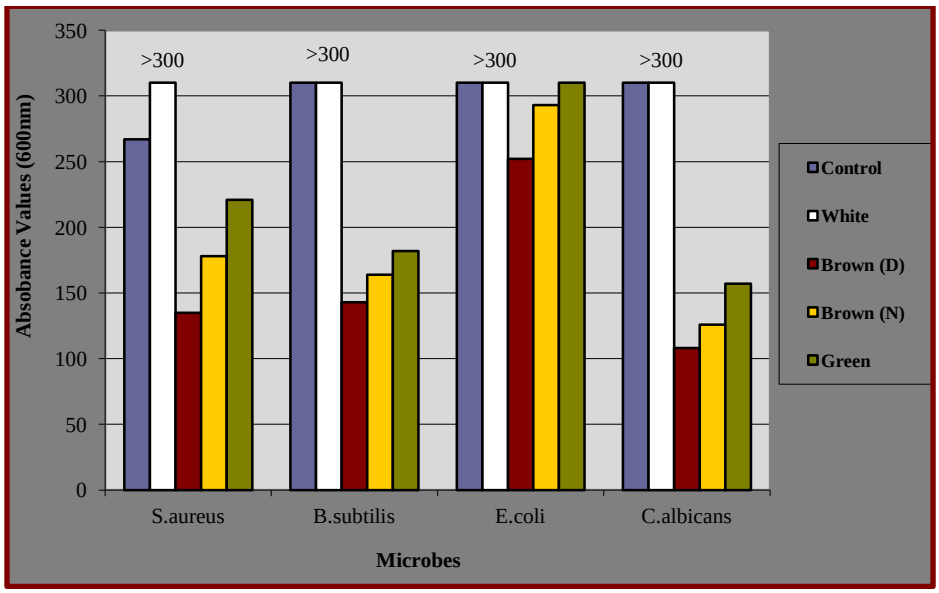


FIGURE 3.2: COMPARATIVE ANALYSIS OF COLONY FORMING UNITS BY COTTON SAMPLES

To further substantiate the results at the end of evaluation tests of performance properties, a statistical analysis of the results was carried out by SPSS (15 version) statistics program with a 95% confidence interval. The variation analysis (ANOVA) indicated that the different cotton samples exhibited significantly different behavior with the selected microbes (Table 3.3).

TABLE 3.3: VARIATION ANALYSIS OF DIFFERENT COTTON SAMPLES

Microbe	Sum of Squares	DF (Degree of Freedom)	Mean Square	F value	Significance Level
S. aureus	0.018	8	0.002	21.550	0.000

B. subtilis	0.008	8	0.001	9.043	0.006
E. coli	0.003	8	0.000	45.356	0.000
C. albicans	0.041	8	0.005	604.210	0.000

It can be thus be said because each variation source (different cotton samples) relating to the selected four microbes had a highly significant level value below 0.001 in most cases.

Table 3.3 indicates that between group variation was very high (as indicated by F value) especially in case of C. albicans followed by E. coli, S. aureus and B. subtilis. Hence it can be concluded that different cotton samples exhibited significantly different microbial activity with the selected microbes.

TABLE 3.4: VARIATION ANALYSIS (WITHIN GROUP) OF DIFFERENT COTTON SAMPLES WITH VARIOUS MICROBES

Dependent Variable	Sample (I)	Sample (J)	Mean Difference (I-J)	Standard Error	Significance Level
S. aureus	WM	BD	-0.22600*	0.038726	**0.002**
		BN	-0.18433*	0.038726	**0.006**
		GN	0.02467	0.038726	0.917
B. subtilis	WM	BD	-0.07467	0.025673	0.076
		BN	-0.03033	0.025673	0.215
		GN	0.05533	0.025673	0.654
E. coli	WM	BD	-0.18067*	0.015864	**0.000**
		BN	-0.06900*	0.015864	**0.001**
		GN	-0.10900*	0.015864	**0.010**
C. albicans	WM	BD	1.92467*	0.058265	**0.000**
		BN	1.36267 *	0.058265	**0.000**
		GN	-0.12500	0.058265	0.218

Table 3.4 gives the variation analysis of within group of different cotton samples. When the microbial behavior of naturally coloured cotton samples was compared with conventional white cotton, significant level of difference was observed with all dependent variables (microbes). Naturally brown cotton samples (BD and BN) showed significant level of inhibition against all microbes except for *B. subtilis*. Between the two brown varieties, difference in inhibition property was observed. The brown sample BD from Dharwad exhibited highly significant level values (below 0.001) of inhibition with fungus *C. albicans* and Gram negative bacteria, *E. coli*. Inhibition with Gram positive bacteria *S. aureus* and *B. subtilis* was also higher in case of brown BD variety. This difference could be attributed to the presence of higher heavy metal ion content than brown BN variety.

Naturally green coloured cotton sample showed significant difference in level of microbial inhibition (below 0.05) with Gram negative bacteria, *E. coli* as compared to conventional white cotton. Green sample GN also showed difference in inhibition with fungus *C. albicans* followed by *B. subtilis* and least resistance to *S. aureus* (Table 3.4).

Therefore, it can be concluded that naturally coloured brown and green cotton are ideal alternatives to white cotton as they inhibit the growth of microbes and check infections from setting-in. Amongst the naturally coloured cotton samples, brown samples have higher inhibition towards the selected microbes due to the presence of catechin and other tannin derivatives which are absent in green coloured cotton. Brown BD variety exhibited higher microbial inhibition than brown BN variety probably due to presence of higher metal ion content.

IV. CONCLUSION

The performance properties like antimicrobial for naturally coloured cotton was assessed in order to explore and determine its special inherent properties which would help increase consumer acceptance and augment their production and consumption. The antimicrobial property of conventional white and naturally coloured cotton was assessed through Optical density and Standard Plate Count Test. The analysis of variance (ANOVA) highlighted that naturally coloured cotton significantly resisted the growth of microbes *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans*. Amongst the coloured cottons, brown sample gave higher resistance due to the presence of catechin and other derivatives. Catechin attacked the cell membrane and caused hydrolysis of conidia and hyphae present in fungus. It also resisted bacterial growth by damaging the bacterial membrane. Gram positive bacteria were better resisted as they are more sensitive to the bactericidal effect of tannins present in naturally coloured cottons.

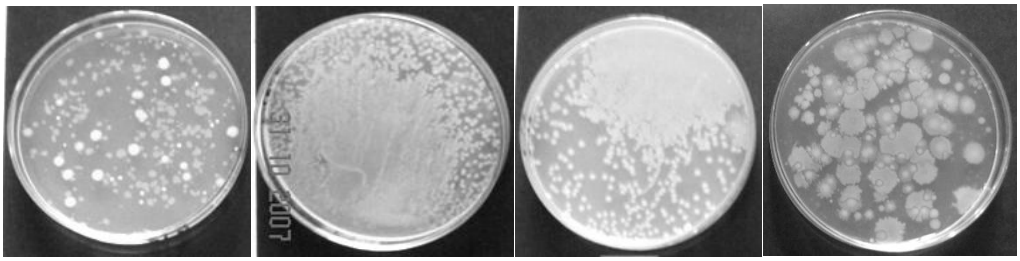
PLATE 3.1

COMPARATIVE ANALYSIS OF COLONY FORMING UNITS (CFU) OF COTTON SAMPLES (Dilution 10^{-6})

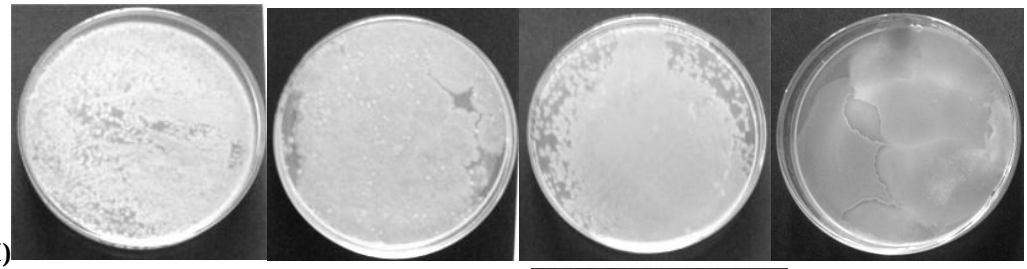
MICROBE ⟶ ***S.aureusB.subtilis E.coliC.albicans***

SAMPLE ↓

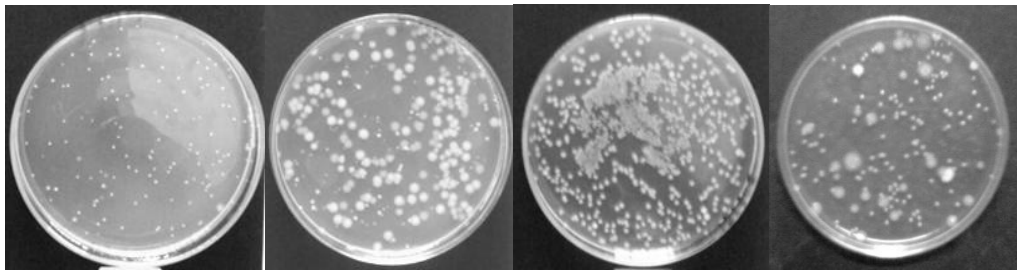
Control



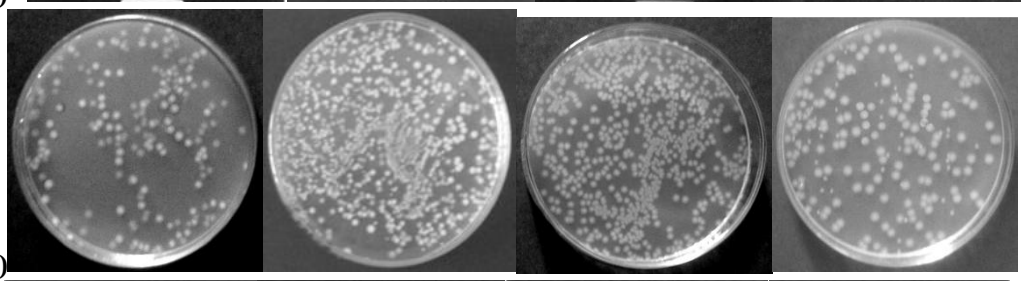
White(WM)



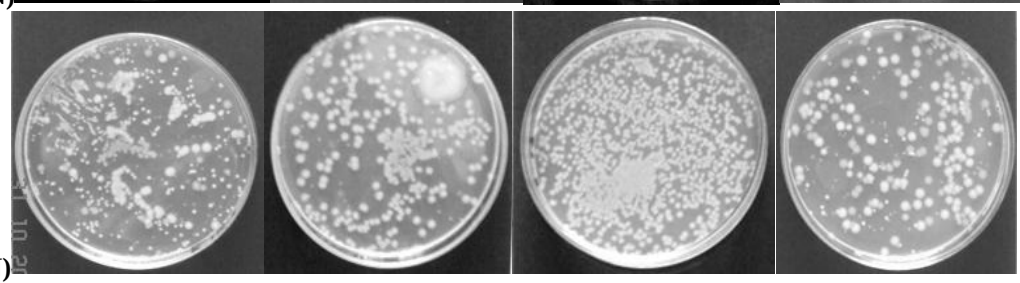
Brown(BD)



Brown(BN)



Green(GN)



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