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Microbial pigment as an alternative to synthetic dye

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Abstract : *The present study aimed at evaluating the dyeing potential of microbial pigments for different fabrics like silk and cotton and to develop an eco friendly dye for textiles dyeing. In the present study, total of six pure cultures of bacterial and fungal strains were isolated from different samples on NA, PDA and MSA media. Pigment producing isolates were mass cultivated for the production of pigments and further analysis. The pigment production was screened by taking O.D. at regular time intervals during the incubation period and pigments were extracted by solvent extraction method. Fungal isolates, isolate 3(Aspergillus spp.) and 4(Penicillium spp.) produced green and reddish brown colored pigment respectively and bacterial isolates, isolate 1(Streptococcus spp.),2(Bacillus spp.),5(Staphylococcus spp.) and 6(Pseudomonas) produced yellow, brown, dark yellow and green colored pigments respectively. These pigments were applied to fabric and tested for percentage absorption. It was found that pigments showed high affinity for silk fabrics as compared to cotton fabrics. The pigment of Penicillium showed maximum absorption percentage (65.52%) followed by Aspergillus (63.78%), Pseudomonas (55.02%), Bacillus (48.37%), isolate 1(32.66%) and Coccus (31.08%). It has been found that the percentage absorption was more in mordanted fabric as compare to unmordanted fabric.*

Key words*: Mordants, pigments, natural dye, synthetic dye, FT-IR*

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Introduction :

Colors are one of the most significant visual properties of textiles, food and cosmetics. The textile industry is one among the rapidly growing industries worldwide. In India it accounts for 14% of the total industrial production and contribute to nearly 30% of the total exports .Textile industry is one of the major industry which uses dye in large quantity. Textile industries utilize enormous amount of synthetic dye and consequently these synthetic dye make the textile effluent hazardous for the environment (Kumar et al., 2015).

Dye is a natural or synthetic substance which is used to add a color to textile. The presence of a substance known as chromophore in the dyes gives color to the textile. Synthetic dye is a type of organic dyes originally derived from coal-tar derivatives, petroleum by-products and earth minerals. Natural colorants are the term used for all the dyes and pigments derived from natural sources like plants, animal and microbes. Natural dyes and pigments have emerged as an important alternative to potentially harmful synthetic dyes (Velmurugan et al., 2010). The Production of pigments from microorganisms is beneficial over other sources because microorganisms can grow rapidly which may lead to a high productivity of the product. The production of synthetic colors is economically efficient but they are facing challenges like dependence on non-renewable oil resources, environmental toxicity, and human health concern.

Biocolor consist of two words 'bios' means natural and color means anything that impart color or anything which is used for coloring purpose. So, all biocolors are natural pigment or natural dyes which emerged as an alternative of potentially harmful synthetic dyes. Increasing consumer awareness put emphasis on the production of biocolors or natural colors extracted from fruits, vegetables, roots and microorganisms. Natural dyes are non toxic, non-polluting and less health hazardous, and their antioxidant and antimicrobial effect adds to their positive effect (Sharma et al., 2012).

Microorganisms produce various pigments like carotenoids, melanins, quinones, flavins, prodigiosins and more specifically monascins, violacein (Gupta et al., 2011). These microbial pigments are use as biocolorants because of

Easy propagation and wide range of strain selection, High versatile nature, Fermentation is faster and more productive production compare to other chemical processes, Easy to manipulate genes, Cheap substrate for bulk production, Extraction of pigment using simple liquid-liquid extraction technique minimizes operation cost.

So, there is an urgent need for alternative colorants that are natural, cost effective and easily biodegradable and without production of recalcitrant intermediates when they enter the ecosystem. Hence there is an increasing interest involving microorganisms as a possible alternate source of colorants used in textile industry (Sharma et al., 2012).

Materials and Methods :

Samples were collected from local areas. Soil sample was collected from the campus of Patna Womens's College, Kankarbagh Colony and Patna City using sterile spatula and aseptically transferred into sterile polythene bag and stored under refrigeration until the analysis were carried out.

Soil samples and milk samples were serially diluted upto 10^{-7} for bacteria and 10^{-3} for fungi and were spread on NA and PDA plates and were incubated at 37°C and 26 °C respectively. Each culture plate was examined for colored colonies and streaked on respective media.

Bacterial isolates were characterized by their colony features, morphology, microscopic characteristics, colors of colonies and by biochemical tests such as catalase, oxidase, coagulase etc. The Fungi isolates were identified based on the appearance, mycelia, spores and color and microscopic view.

Fungal cultures were mass cultured in Potato Dextrose Broth (PDB). Fungal isolates were inoculated in 100ml PDB and in one flask a pinch of copper sulphate was added to increase the pigmentation and incubated at 28ºC in a static condition for 3-4 weeks for pigment

production(Aishwarya and Anchana Devi, 2014). Bacterial isolates were inoculated in Nutrient Agar (NA) Broth and incubated in shaker incubator for 24h at 30ºC for inoculums preparation. After 24 h, 1% of the cell suspension was used as inoculums and these inoculums were inoculated in NA broth and kept at 30ºC for 10-15 days in static condition (Raju and Radha et al. 2015).

The microbial isolates in broth cultures were tested for the production of the colors by taking the optical density of the pigment at different absorbance according to their colors (Aishwarya and Devi, 2014).

Pigment extraction was done by solvent extraction method. The fungal mycelium was harvested, and soaked in solvent and well grounded using mortar and pestle. After removal of mycelial mat, the remaining was centrifuged at 3000 rpm for 20 min and supernatant was mixed with 95% ethyl alcohol (5ml supernatant in 6ml of ethanol) and kept in shaker for 30 min which resulted in uniform mixing. This mixture was centrifuged at 3000rpm for 15min and colored supernatants were obtained which was used for textile dyeing (Velmurugan et al., 2010).

The bacterial cultures were centrifuged at 6000rpm for 10min and supernatant and pellets were dissolved in equal amount of both acidified alcohol and chloroform in separate flask and again centrifuged at 6000rpm for 10min. Pellet was discarded and supernatant was used for dyeing of textiles (Goswami and Bhowal, 2014).

The FT-IR spectra of extracted pigments were recorded to identify the presence of different functional groups in pigment. One drop of extracted pigment was added in 200mg of potassium bromide (KBr) to form dry pellet of pigment and then these pellets were analyzed in the range of 400-4000 cm^{-1} (Chakraborty et al., 2015).

Broths inoculated with isolate 5 were kept at different temperature (30ºC, 37ºC & 60ºC) to study the relationship between temperature and pigment intensity. The production of the pigment were estimated after incubation (Prasad et al., 2013).

Cotton and silk fabrics were washed in a solution containing 1g of commercial detergent in 50ml of distilled water at 50^{0} C for 25 min. The scoured material was thoroughly washed with tap water and dried (Poorniammal et al.,2013).

A mordant is a substance used to set dyes on fabrics. Mordanting was done by using different mordants like ferrous sulphate, potassium dichromate, and ecofriendly salts and vinegar were also used to reduce the use of chemical mordants. 1-3% of ferrous sulphate and potassium dichromate were used as mordants. The scoured clothes were treated with mordants using a conical flask at 60°C for 30minutes with mordant to liquid ratio 1:20 and the temperature was maintained constant. After mordanting they were washed and dried. Vinegar was mixed with water in the ratio of 1:4 and the scoured fabrics were boiled in this solution for 45min.After this the fabrics were directly used for dyeing.Salt was mixed with water in the ratio of 1:6 and the scoured fabrics were boiled in this solution for about 1h. The mordanted fabrics were washed thoroughly to remove salts and then dyed (Gupta et al., 2011).

Dyeing was done on cotton and silk fabrics. Dyeing was done by putting the cloths in flask containing extracted dye and kept at 45ºC for 10 min and the temperature was raised to 80ºC for 45 min. The dyed cloths were then washed and dried in sunlight and then evaluate for color fastness properties (Devi and Karuppan, 2015).

Effect of different mordants, temperature and time duration on dyeing was also observed.

The color fastness is the ability to resist the color change or transfer in the material when it is in contact with other material or in presence of sunlight, water (Samanta and Agarwal, 2009). Color fastness of the dyed fabrics was tested by washing the fabrics with mild detergents (wash fastness), Rubbing the dyed fabrics with undyed white fabrics (rub fastness), drying the dyed fabric in sunlight (light fastness).

According to Aishwarya and Anchna Devi (2014) , percentage absorption of the dyed fabrics was calculated on U.V. spectrophotometer at different wavelengths. The percentage was determined for both mordanted and unmordanted dyed fabrics separately using the following formula:

$$\text{Percentage absorption (\%)} = \frac{\text{O.D before dyeing - O.D after dyeing} \times 100}{\text{O.D before dyeing}}$$

Results and Discussion :

Total of six microbial isolates were isolated from different samples. Out of these six microbial isolates, four isolates of bacteria (isolate1,2,5 and 6) and two isolate of fungi (isolate 3 and 4) were isolated. On the basis of the mentioned characteristics (Table 1) the isolated fungal strains were of *Aspergillus* (isolate 3) and *Penicillium* (isolate 4).

On the basis of the mentioned characteristics (Table 2) and biochemical results bacterial isolates identified as Isolate 1 belong to *Streptococcus* species, Isolate 2 belong to *Bacillus* species, Isolate 5 belong to *Staphylococcus species* and Isolate 6 belong to *Pseudomonas* species.

The bacterial isolates (isolate 1, 2 5, and 6) showed pigment production in Nutrient broth. Isolate 1 *(Streptococcus spp.)* produced light yellow colored pigment in NA broth. Isolate 2 produced brown colored pigment in culture broth. Likewise, production of pigment by *Bacillus* species was reported by Goswami and Bhowal (2014).

Yellow colored pigment was produced by Isolate 5 in broth and it was reported by Chintapenta et al., (2014) that some species of *Staphylococcus* produces saphyloxanthin pigment which are yellow in color. Isolate 6 produced green colored pigment in broth. *Pseudomonas* species produces pyocyanin, a green colored pigment reported by Sudhakar et al.,(2015).

The fungal isolates (isolates 3 and 4) showed pigment production in potato dextrose broth. Isolate 3 (*A.niger*) produced brown pigment at stationary phase. Similarly, Aishwarya and Devi (2014) also extracted brown pigment from *Aspergillus spp*. Isolate 4 shown reddish brown coloration in stationary phase. Chintapenta et al.,(2014) also extracted red pigment from mangrove *Penicillium.*

The greater value of O.D. of the sample as compared to the blank indicated the presence of pigment in the sample. An increase in the O.D within the incubation period was observed which demonstrated that pigment production increased simultaneously. Similar result was reported by Velmurugan et al., 2009 (Fig 1).

Pigment was extracted by solvent extraction method. Different types of solvent were used which give different colors as pigment 1 (yellow), pigment 2 (brown), pigment 3 (green), pigment 4 (reddish brown), pigment 5 (yellow), pigment 6 (green) (Table 3 and Fig 2).

FT-IR analysis for different pigments are shown in Figs 3,4,5,6,7 and 8.The FT-IR spectrum report was analyzed and interpreted corresponding to the standard peak values. In pigment 1 the peaks at 2924 and 2854 represents the methyl groups (-CH) stretching, 1635.22 as hydroxyl (-OH) stretching, 1406.16 as –OH bend and 1380 as CH_3 C-H bend.

In case of Pigment 2, peaks at 2827.03 cm^{-1} represents the O-H stretching,1634.38 cm^{-1} as C=C conjugate,1588.57 cm^{-1} as N-H bend and 1300.51 $cm^{-1}$and 1334.13 as as C-F stretch while in pigment 3 peaks at 1633.25cm^{-1}represents as C=C stretch, 1384.13 cm^{-1} as CH_3CH bend and 1350.58 cm^{-1} as C-F stretch. Similarly, Sayyed and Majumder

(2015) reported presence of alkene (C=C) stretch,C=O stretch in pigment extracted from *Aspergillus niger.*

In Pigment 4 peaks at 2812.87 represents as CH stretch, 1632.27 as C=C stretch, 1537.79 as C=C (aromatic rings), 1334.08 as C-N stretch and 767.58 as aromatic C-H bend. Pigment 5 peaks at 2816.12 represent as C-H stretch, 1632.36 as alkene C=C stretch, 1350.58 as CF stretch, 1114.04 as C-O stretch and 985.34 as C-H bend. Pigment 6 peaks at 2820.52 represents as C-H stretch, 1550.35 as N-O stretch, 1380.58 as CH_3CH bend and 788.14 as aromatic C-H bend.

Temperature is a major parameter which governs the growth of microbes as well as pigmentation. With the increase in the incubation temperature, the pigmentation increased. The color of the broth changes as it was light yellow at 30ºC, and with increasing temperature at 37 ºC slight change in color was observed and at 60ºC, the color changed from yellow to dark brown (Fig 9).

Dyeing of fabrics : Extracted pigments were used to dye fabrics. Mordanted fabric showed better and bright coloration than unmordanted fabric. Cotton is a cellulosic fibre which does not have affinity for natural dye so it requires mordants which acts as a link between textile fibre and dye. The colored pigment is highly and easily absorbed on silk fabric and also silk fabric showed better fastness property as compare to cotton fabric. Mordanted fabric showed good fastness property than unmordanted fabric (Sharma et al., 2012) (Fig 10).

It was observed that pre mordanting technique with both natural and chemical mordants imparted better coloration in the fabric. Among chemical mordants ferrous sulphate was found to be more liable than potassium dichromate In natural or ecofriendly mordant, salt was observed to be more liable than vinegar because salt mordanted fabrics showed better coloration than vinegar. As vinegar contains little amount of acetic acid so in some cases it changed the color of the pigment and give different shade of colored fabrics. Similarly, Devi and Karuppan (2015) reported that the dyeing can be improved by using different metal mordants.

Temperature is the major parameter in dyeing process. It was observed that at temperature 30ºC pigment absorption was very less and with the increase in temperature, pigment absorption in fabric also increased. The optimum temperature for dyeing was observed between 60ºC to 75ºC (Poorniammal et al., 2012).

The result showed that the uptake of pigment increased with increase in time. The samples required maximum 60 min for dyeing and hence 60 min was concluded to be the optimum time duration for dyeing of fabric. An overall study showed that silk fabric required less time as compared to cotton fabric (Poorniammal et al.,2012)

During the present study color fastness properties (wash fastness, rub fastness and light fastness) of dyed cloth was performed. It was found that the mordanted fabric show good fastness property as compare to unmordanted fabric.

The mordanted fabrics showed higher percentage of dye absorbance than unmordanted fabrics. Silk showed higher affinity as compared to cotton in both unmordanted and mordanted form. Pigment 4 showed maximum absorption percentage (Silk-65.52% and Cotton-64.34%) followed by pigment 3 (Silk-63.78% and Cotton-57.94%), pigment 6(Silk-55.02% and Cotton-53.60%), pigment 2 (Silk-48.37% and Cotton-44.58%), pigment 1(Silk-32.66% and Cotton-25.68%) and pigment 5(Silk-31.08% and Cotton-28.15%). The overall result showed that the mordanting is necessary in dyeing and silk has more affinity for microbial pigment as compared to cotton (Fig 11).

Conclusion :

The present study revealed that beside plants, microbes (like bacteria and fungi) are also capable of producing pigments which gave different shades

of color when applied to fabrics (cotton and silk). Thus, it can be concluded that microbial pigment could serve as a safer alternative for dyeing in textile industries.

In the present study, different microbial strains were isolated from soil and were screened for the presence of pigment. The bacterial and fungal isolates were mass cultivated in respective broths. It has been seen that the pigment production by these microbial isolates were influenced by surrounding temperature. These pigments were extracted by liquid-liquid extraction method in which different solvents like ethanol, chloroform, were used. The solubility of these pigments in different solvent varies upon the microbial species. The overall study showed that the microbial pigments were easily soluble in ethyl alcohol as compared to chloroform.

Moreover, it was also found that the dyeing of fabrics also required appropriate temperature and mordants and these mordants acted as a link between fabric and dye. Natural mordants like salt and vinegar were used as an alternative of chemical mordants like ferrous sulphate but natural mordants were found to be less effective than chemical mordants. It has been seen that the intensity of color on fabric was high in silk as compared to cotton fabric. The mordanted fabrics showed better fastness properties than unmordanted fabrics.

There are many more microbes which are yet to be studied for their property of pigment production. Further research in this field is vital to upgrade the range and quality of the pigment in order to completely curb the hazardous effect of synthetic dyes and thus, bring a revolution in the textile industry.

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Table 1. Morphological characteristics of isolated fungi

Isolate no.	Colony characteristics			Source	Organism	Pigment
	Color	Margin	Texture			
3	Black	White	Powdery	Soil	*Aspergillus spp.*	Brown, Green in presence of $CuSo_4$
4	Olivaceous green	White	Powdery	Soil	*Penicillium spp.*	Reddish brown

Table 2. Morphological characteristics of isolated bacteria

Isolate no.	Colony characteristics			Source	Gram's	Oragnism	Pigment
	Color	Margin	Texture				
1	Light orange	Smooth	mucoid	Soil	Positive	*Strepto-coccus*	Light yellow
2	White	Smooth	mucoid	Soil	Negative	*Bacillus*	Brown
5	Yellow	Smooth	mucoid	Milk	Positive	*Staphylo-coccus*	Yellow
6	Light green	Smooth	mucoid	Soil	Negative	*Pseudo-monas*	Green

Table 3: Extracted pigment by solvent extraction method

Isolate No.	Solvent use	Pigment color
1	95% acidified ethanol	Light yellow **(pigment 1)**
2	95% acidified ethanol	Brown **(pigment 2)**
3	95% Ethanol	Brown; greenish ($CuSo_4$) **(pigment 3)**
4	95% Ethanol	Reddish brown **(pigment 4)**
5	95% Ethanol	Yellow, **(pigment5)**
6	Chloroform	Dark green, **(pigment 6)**

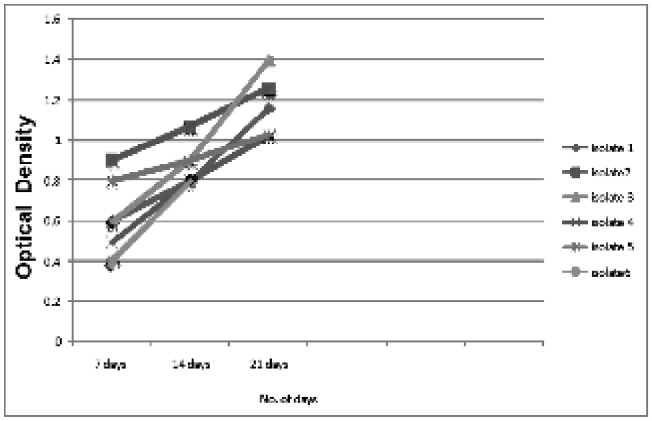
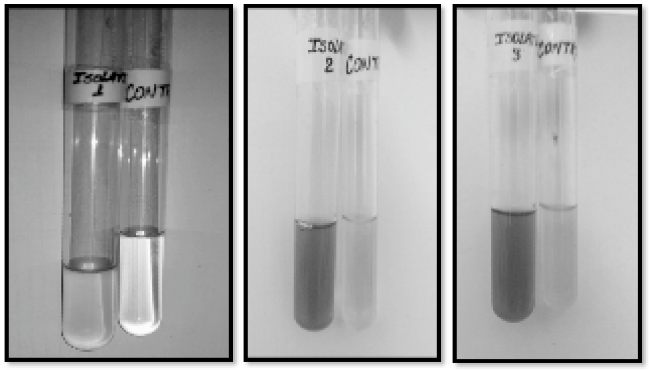
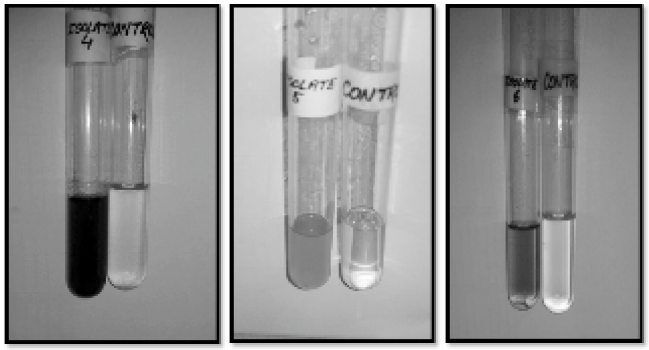


Fig. 1. O.D. of microbial culture recorded over period of 21 days



(a) Isolate 1 (b) Isolate 2 (c) Isolate 3



(a) Isolate 4 (b) Isolate 5 (c) Isolate 6

Fig. 2. Extracted Pigments

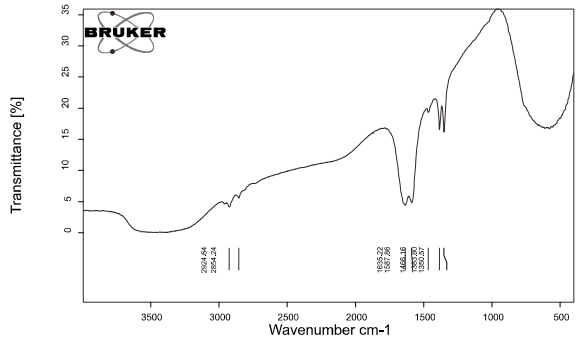


Fig. 3. FT-IR spectrum of pigment 1

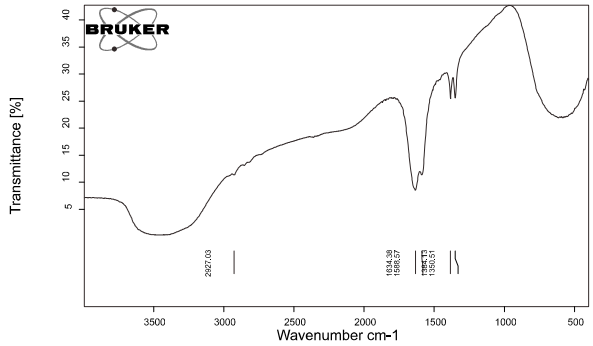


Fig. 4. FT-IR spectrum of pigment 2

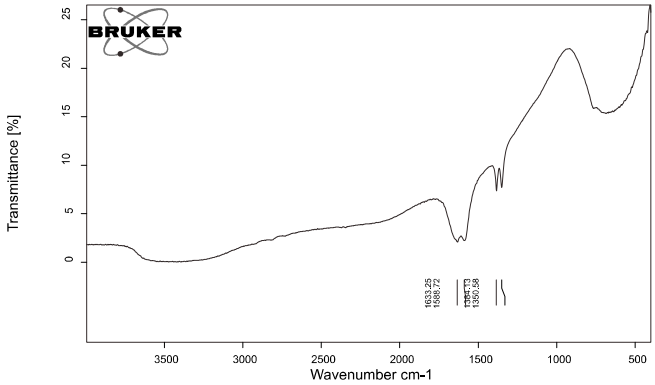


Fig. 5. FT-IR spectrum of pigment 3

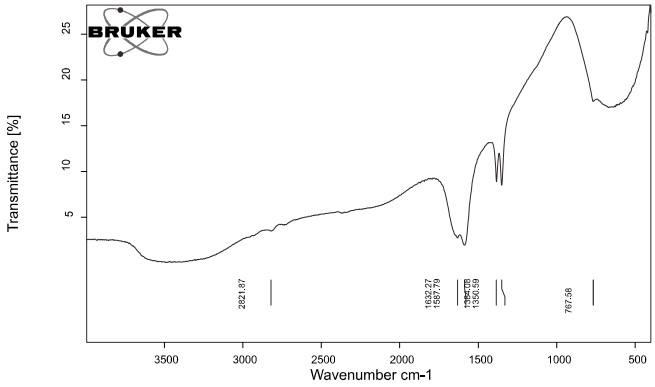


Fig. 6. FT-IR spectrum of pigment 4

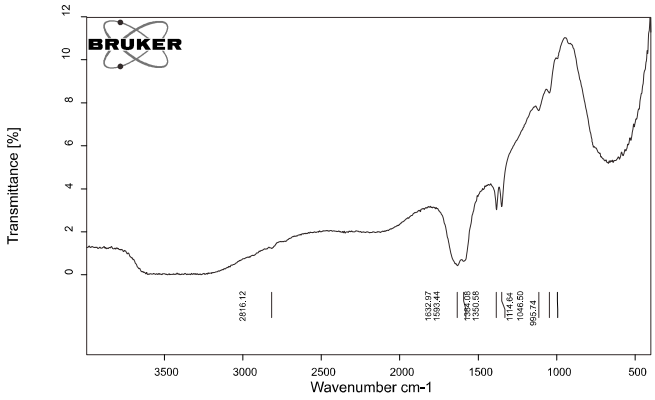


Fig. 7. FT-IR spectrum of pigment 5

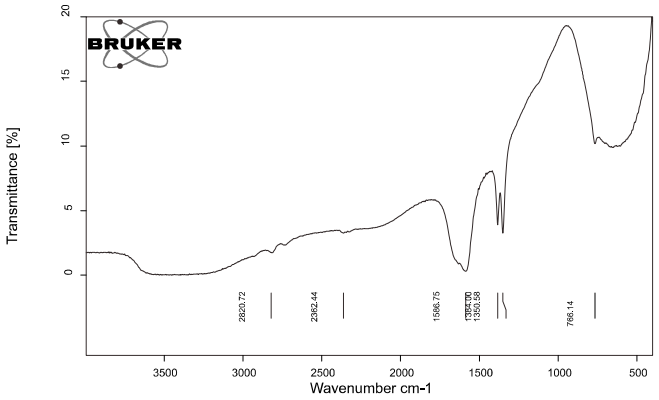


Fig. 8. FT-IR spectrum of pigment 6

Fig. 9. Color changes at different temperature

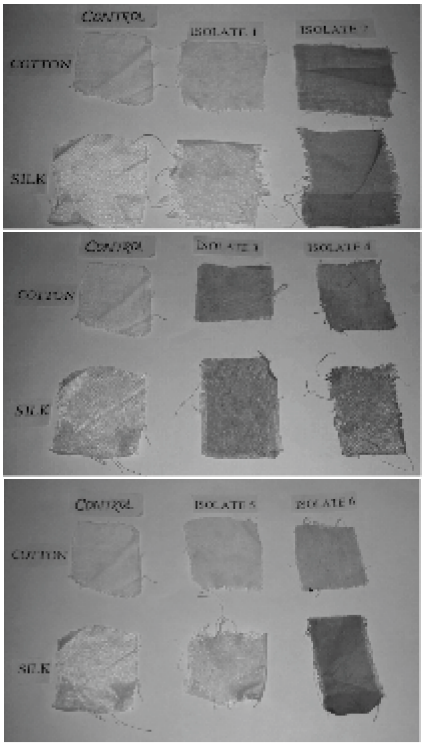


Fig. 10. Dyed fabric samples

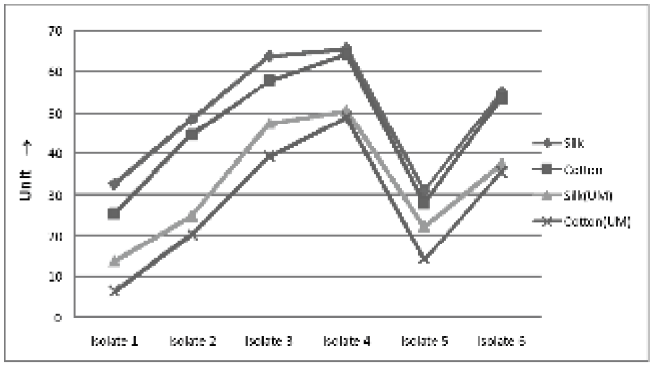


Fig. 11. Pigment absorption in mordanted and unmordanted fabric

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