Chapter - 4

Fungal Extracellular Pigments
4. Fungal Extracellular Pigments

4.1 Introduction

The worldwide demand for food and textile colourants is rapidly increasing and they are important determinants for acceptability from the public. For imparting pleasing and attractive colours to food and textile, many natural colours have been in use since ancient times (Francis, 1987). There are many plant materials that can be used for dyeing yarns and materials like roots, bark, leaves, berries, seeds, twigs, branches, tubers, and nut hulls, each capable of producing a range of colours with various mordants. In addition, when properly applied, natural dyes are fast, resisting was fading due to exposure to sunlight (David, 1980).

Many companies have decided to utilize natural pigments mainly from plant and animal sources. However, these additives have numerous drawbacks such as instability and low water solubility, and are often not available throughout the year when compared to microbial pigments are of industrial interest (Jiang et al., 2005; Gunasekaran and Poorniammal, 2008; Mendez et al., 2011).

Several attempts have so far been made to evaluate the techno-economic feasibility of today’s alternative dye crops. Among the species examined, common madder (Rubia tinctorum L), woad (Isatis tinctoria L) and weld (Reseda luteola L) proved to be quite interesting sources of red (alizarin), indigo (indigotin) and yellow (luteolin) dyes respectively, either for their agronomic characteristics or for their dyeing properties (Marotti, 1997). In fact, all three dyes were extensively exploited until the commercial success of their synthetic analogues (Ball, 2002). The main disadvantage of these natural dyes lies in the order of magnitude of their extraction yield factors. To
overcome this limitation, it was suggested to exploit the potentiality of other biological sources such as fungi (both moulds and yeasts), bacteria, algae and plant cell cultures, since appropriate selection, mutation or genetic engineering techniques are likely to improve the pigment production yields significantly with respect to wild organisms (Santis et al., 2005).

Mushrooms have been regarded as popular folk or effective medicines used to treat various human diseases, such as hepatics, hypertension and hypercholesterolemia, and their biochemical potential and their adaptation to extreme life conditions in liquid media have been exploited to produce useful substances such as antibiotics, enzymes, organic acids and pigments (Cho et al., 2002). Monascus red pigments are of polyketide origin and are used commercially in the orient as non-toxic colourants for colouring rice wine, "Koji", soybean, cheese and red meat. The red pigments have attracted worldwide commercial interest, but little information is available on the production of this pigment by other microbial sources. It has been reported that Penicillium may be the potential candidate to produce polyketide structure compounds (Juzlova et al., 1996; Jiang et al., 2005).

Characteristic pigments are produced by a wide variety of fungi. Species of Drechslera produced hydroxyanthraquinones (e.g., Helminthosporin - maroon, brown), catenarin (red), cynodontin (bronze) and tritisporin (red brown). A red-violet pigment was isolated from Rissula vinosa. Dark brown or black pigments occur widely in fungi. Except for reactions to wounding, melanogenesis in fungi is restricted to certain developmental stages in special structures such as chlamydospires or microsclerotia (Thielaviopsis verticillium), conidia (Aspergillus niger), and hyaline mycelium. In the Dematiaceae, both hyphae and conidia were heavily pigmented (Alternaria, Curvularia, Drechslera) (Margalith, 1992).
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Fungal extracellular pigments

Natural colourants are considered to be safer than synthetic ones and their applications in foods, cosmetics and pharmaceuticals are growing rapidly. There are a number of natural pigments, but only a few are available in sufficient quantities for industrial production. Production of pigments from microorganisms is advantageous over other sources because microorganisms can grow rapidly which may lead to a high productivity of the product (Lauro, 1991; Kim et al., 1999). In food industries they are used as additives, colour intensifiers, antioxidants, etc., textile industries used for textile dyeing and also in the manufacturing of cloths with antimicrobial properties for example anthraquinones. Pigments come in a wide variety of colours and some are water-soluble. For these reasons, many of these compounds have been produced, isolated, and characterized (Duran et al., 2002).

Polyketides are synthesized by the catalytic action of the multifunction enzyme polyketide synthases (PKSs). Polyketides can be classified on the basis of their biosynthesis indicating the number of “C2-units” that have contributed to the polyketide chain and according to the type of cyclization the precursor has undergone. The terms triketide, tetraketide, pentaketide, etc., denote compounds derived from three, four or five “C2-units” respectively (Turner, 1971). Fungal polyketide pigments represent tetraketides to octaketides and some of them involve mixed biosynthesis meaning that they involve other pathways (such as amino acid, or terpenoids) in addition to the polyketide pathway. They typically represent anthraquinones, hydroxyanthraquinones, naphthoquinones and azaphilone structures. Role of polyketide pigments is still not specifically known but the following information would give a fair idea about the diverse roles they could play for their producers. Many of the polyketide pigments have an antibiotic activity; therefore, their production confers upon the organism an advantage in its natural environment.
4.2 REVIEW OF LITERATURE

Natural products are synthesized from the plants, animals and filamentous fungi, because they have an ecological function and are of value to the producer. Depending on the type of compound, they serve different functions varying from a protective action against lethal photo-oxidations (carotenoids) to protection against environmental stress (melanins) and acting as cofactors in enzyme catalysis (flavins). Besides providing functional diversity to the host, these pigments exhibit a unique structural and chemical diversity with an extraordinary range of colours (Mapari et al., 2005).

Water soluble pigments or dyes are of fungal origin, they have great biotechnological interest. *Monascus* is a typical ascomycete that produces a cleistothecium, a closed fruiting body containing eight ascospores, but reproduces asexually by the formation of conidiospores and a vegetative mycelium. The pigments may appear both in the mycelium (intracellular) and in the fermentation broth (extracellular). It was suggested earlier that microbial pigments having no obvious function, they should be classified as secondary metabolites, but many published data showed in a special cases that the pigments are certainly not a secondary metabolite (Duran et al., 2002). The main components of *Monascus* pigments are a series of azaphilone compounds and their N-substituents, such as monascorubramine and rubropunctamine (red-purple), monascorubrin and rubropunctatin (orange), and monascin and ankaflavin (yellow). Special attention has been focused only on the strains belonging to the *Monascus* genus of filamentous fungi by earlier workers, but later some other authors referred to these fungi as potent producers of natural pigments (Tseng et al.,
2000; Carvalho et al., 2003). One of the most important are Monascus pigments, which have been used for centuries as food colours in eastern countries, and which present potential use in meats, beverages, sauces and soups.

Production of water-soluble pigments directly by the fermentation process offers a more acceptable alternative to the chemical semi-synthetic processes, since it avoids the use of chemical additives in foods (Spears, 1988) and pigments produced by an alternative route through the application of biotechnological tools, the microorganisms like microalgae and several classes of fungi are known to produce a wide range of excreted water-soluble pigments, but they have low productivity (Mapari et al., 2005).

Ascomycetous and hyphomycetous fungi are more suitable for biotechnological production because they can be grown in a relatively easier way to give high yields using existing culture techniques. Food colourants from ascomycetous fungi have been explored with few successful attempts. Carotenoids such as β-carotene and lycopene have been known to be produced by fungal cell factories. The successful industrial production of β-carotene by Blakeslea trispora is the best example to be given. Other sources are Mucor circinelloides (zygomycete fungus), and Phycomyces blakesleeanus (Mapari et al., 2006). Pigments like catenarin, chrysophanol, cynodontin, helminthosporin, tritispore and erythroglaucean are produced by Eurotium spp., Fusarium spp., Curvularia lunata and Drechslera spp. The red colourant is an extracellular metabolite of the anthraquinone class and is claimed to be produced by a variety of Penicillium oxalicum. Non-carotenoid pigments exhibit a broader colour range when compared with the limited colour range of carotenoids and as these pigments are water-soluble, they do not require chemical modification or the use of carriers and stabilizers.
for dispersion in foods. *Penicillium marneffei* produces large amounts of extracellular red pigments and one of the pigments was identified as monascorubramine, the red Monascus pigment (Mapari *et al.*, 2005).

Cho *et al.*, (2002) reported that, there are other microorganisms other than *Monascus* genus, which have the ability to produce pigments in high quantities, such as those belonging to the genus *Paecilomyces*, producing red, yellow, and violet pigments in quantities of up to 4.73 g/L. Microorganisms belonging to the genera *Aspergillus* and *Penicillium* have also been studied as potential producers of natural pigments (Engstrom *et al.*, 1982; Suhr *et al.*, 2002; Jiang *et al.*, 2005; Dufosse, 2006; Zavala *et al.*, 2007; Rivera *et al.*, 2008). The production of *Monascus*-like pigments from *Penicillium* strains has recently been reported that, these pigments have a potential use in the food industry because they are not associated with citrinin production. They are homologues of pigments of *Monascus* which have similar chromophore polyketides (Mapari *et al.*, 2008a) and of fungal strains of the species *Epicoccum nigrum* that produce yellow pigments (Mapari *et al.*, 2008b).

According to Mendez *et al.*, (2011) during the production studies of fungal pigments metabolically, the effects of pH and temperature are associated with changes in the activities of proteins, so the culture conditions can control some activities such as cellular growth, production of primary and secondary metabolites, fermentation, and the oxidation processes of the cell. Velmurugan *et al.*, (2010) reported that, liquid state fermentation under different conditions of illumination, using the blue 492–455 nm, green 577–492 nm, yellow 597–577 nm, red 780–622 nm, white and darkness for the extracellular pigment production from the filamentous fungi. The absorption spectra of
the pigments extracted from different coloured light and darkness indicated that pigment composition largely changes depending on the light and its conditions. Incubation in the total darkness resulted in increased pigments production, followed by red, blue, unscreened white light, green and yellow in extracellular pigment yield in all the isolates.

According to Ma et al., (2000) recent clinical observations now clearly shown that, the red yeast rice has ability to lower blood-lipid levels in animal models and in humans and this observation is partly due to the presence of cholesterol synthetase inhibitors (HMG-CoA reductase inhibitors). To understand the health-related properties ascribed to red yeast rice, they were undertaken complete study of the metabolites of red yeast rice (Monascus purpureus). Their study reports that the isolation and identification of seven monacolin analogues from red yeast rice, so due to the presence of these compounds it may explain in part the cholesterol lowering ability associated with this traditional Chinese food. Red yeast rice contains substances, such as a group of antihypercholesteromic agents, including monacolin K and the hypotensic agent L-aminobutyric acid and antibacterial compounds (Li et al., 2004). The evolution of highly resistant bacterial strains has compromised the use of newer generations of antibiotics. Several microorganisms like Monascus, Peacilomyces, Serratia, Cordyceps, Streptomyces and Penicillium have the ability to produced pigments in high yield, which have been developed and used to treat the wound infections and skin diseases caused by the pathogens. Fungal pigments may be a better source of antimicrobial compounds than synthetic drugs, therefore, the investigations of the antimicrobial activity of natural products have opened new ways for drug development in the control of antibiotic resistant pathogens (Visalakchi and Muthumary, 2009).
Recent studies were reported about the manufacturing of textiles coated with antimicrobial compounds. In the textile industrial sectors, there has been increasing interest in the manufacture of clothing products especially under garments coated with anti-bacterial components. Now a day consumers are looking for clothing products, which should provide greater comfort and remain fresh and odour-free in use. Clothing of textile materials can act as carriers for microorganisms such as pathogenic or odour-generating bacteria and moulds. Often leads to objectionable odour, dermal infection, product deterioration, allergic responses and other related diseases, which necessitate the development of clothing products with anti-microbial properties (Velmurugan et al., 2009).

Varieties of antimicrobial textile materials have been reported, including the development of antibacterial nylon fibre by attaching a phosphate glass as an anti-bacterial agent. Multiple chemical surface coatings and chemical reagents have been tried on nylon, cellulose, polypropylene and polyethylene fibres, but many of these approaches lead to environmental and health related problems. Anthraquinone pigments and their derivatives identified from the various species of fungus and lichens exhibit the various interesting biological activities like antibacterial, antifungal, immunomodulatory, teratogenic, cytotoxic and antiprotozoal activities (Duran et al., 1983; Ogihara et al., 2000; Nagia and EL-Mohamedy, 2007). During recent years, many observations have been published with regard to the antioxidant activity of carotenoids, inhibition of mutagenesis, enhancement of the immune response and inhibition of tumor development (Margalith, 1992).
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Fungal extracellular pigments

The use of non-toxic and eco-friendly natural dyes on textiles has become a matter of significant importance because of the increased environmental awareness in order to avoid some hazardous synthetic dyes. In the traditional natural dyeing of textiles an important part of red/yellow dyes was formed by extraction of anthocyanin/flavonoid dyes from fruits and vegetables. An excellent overview of plant sources and application is given by Schweppes, (1992). Mushrooms and lichens have a rich history as sources of pigments for textile colouring. Mycelial extracts of some promising mushrooms such as Chroogomplus vinicolor gives red tints, Bankera violascens gives greens and Collybia ioecephala gives blues. They have a tremendous potential for dying wool and silk fabrics (Maldonado and Ibarra, 2005). However, such fungi are difficult to grow under lab conditions and therefore are not suitable for large scale industrial productions. Natural dyes can exhibit better biodegradability and generally have a higher compatibility with the environment. Dyeing industries were also under increasing pressure to minimize the damage to the environment. So the industries are continuously looking for cheaper, more environment friendly routes to existing dye (Duran et al., 2002). Natural dyeing of cotton fabric has always posed challenges, although silk is easy to dye. Several metallic salts, and biomordants have been used by researchers. Tannins were utilized as mordants to increase the uptake of cationic dyes onto cotton wherein cotton was first treated with tannin extract and then with a metal salt solution prior to dyeing, so as to impart adequate wash and light fastness to the dyed fabrics.

Mordanting is the treatment of textile fabric with metallic salts or other complex forming agents which bind the natural mordantable dyes onto the textile fibers. Mordanting can be achieved by either pre-mordanting, simultaneously mordanting or
post-mordanting (Samanta and Agarwal, 2009). Different types and selective mordants or their combination can be applied on the textile fabrics to obtain varying colour or shade, to increase the dye uptake and improve the colour fastness behaviour of any natural dye.

Synthetic colours are found technically more suitable than natural colours and become popular because the former are known for their fastness, available in a wide range of colours, low cost even at high concentration in low volumes (Pattnaik, 1997). But in the world market, the number of permitted synthetic dyes had declined, since some of them are the sources of skin cancer (occupational), disorders and allergic to man (Francalanci et al., 2001), generates hazardous waste and green house gases during processing and are energy intensive. The scrutiny and negative perceptions of synthetic food pigments by the modern consumer have given rise to a strong interest in natural colouring alternatives (Dufosse, 2006). Natural dyes or pigments produced with chemical modification are claimed to be more stable to heat, light or pH changes (Tezuka and Kashino, 1979; Wong, 1982).

According to Kamel et al., (2009) dyeing of cotton fabrics with anionic dyes (synthetic) such as direct and reactive dyes requires the presence of large quantities of electrolyte to increase dye uptake, resulting in serious environmental problems. As a result of this process, large volumes of wastewater, containing significant amounts of dyes and chemicals are discharged from a typical cotton dye house. So he came to know one method, which avoids this problem. It is the process of cationizing the cotton fibre by using cationic agents, which will increase the colour strength of the dyeing process and improves wash fastness using natural dyes.
In the present study researcher chosen this work by keeping the above all studies in mind that, the isolation of effective extracellular pigment producing fungal species from the forest soil, because it is hotspot of biodiversity, here diversified microorganisms are actively involved metabolically and competing for the food and shelter by secreting some extracellular metabolites, they are toxic to one another. So keeping these points in view that, the researcher concentrated on the fungal pigments, using as antimicrobial agents and natural colouring agents for the textile.
4.3 MATERIAL AND METHODS

4.3.1 Study area and sample collection

Forest soil sample was collected from Bhadra Wildlife sanctuary, Western Ghats of Southern India. Organic soil sample was collected in a sterile polyethylene bags at a depth of 5-10 cm by random mixed sampling method in the forest and brought to the laboratory and preserved in a refrigerator for further use. Bhadra Wildlife Sanctuary is a hot spot biological diversity in the Western Ghats, with a wide range of tree vegetation such as dry and moist deciduous, semi-evergreen and evergreen forests (Champion and Seth 1968).

4.3.2 Screening and isolation of pigment producing fungi

Pigment producing fungi were screened and isolated from the forest soil samples by serially diluting and plating method. One gram of forest soil sample was transferred to the sterile 9 ml saline solution and mixed homogenously, it was considered as 10⁻¹ dilution. Transferred one ml aliquot of the dilution 10⁻¹ to 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ upto 10⁻⁷, from that 10⁻³ to 10⁻⁴ dilutions were selected and inoculam was transferred to the respective plates and pour plate method was followed in triplicates, by using Czapek Dox Agar (CDA) and Potato Dextrose Agar medium (PDA), amended with 30 mg/L of streptomycin sulphate to control bacterial growth. The plates were incubated at 25 ± 2 °C in an incubator for five days. Fungi displaying intense attractive bright colours into the medium were selected and further transferred to fresh PDA and CDA medium for the confirmation of its colour producing ability into the solid medium.
4.3.3 Selection and characterization of pigment producing fungi

Based on the rate of coloured component secretion into the medium with contrast to different colours, the fungal species were grouped into +, less; ++, moderate and ++++, good producers of extracellular pigments. These purified colonies were transferred to 3 sets PDA slants, one set was stored at 4 °C, second set was used for further production studies and third set was used for characterization. Characterization was done by observing cultural and phenotypical characters under microscope and identified with the help of standard fungal identification manuals (Domsch et al., 1980; Subramaniyan, 1983; Gilman, 2001; Nagamani, et al., 2006).

4.3.4 Submerged fermentation (SmF)

Actively growing fungal mycelial discs of 5 mm diameter were inoculated on to sterile 250 ml Erlenmeyer flasks containing 100 ml of Czapek Dox Broth using sterile cork borer for the production studies. Czapek Dox Broth of pH 7.0 in Erlenmeyer’s flask (Judd and Wyeszchi, 1975). Flasks were shaken well and incubated at 25 ± 2 °C in the dark and static condition for 6 weeks without disturbing it (Nagia and EL-Mohamedy, 2007).

4.3.5 Extraction and purification of pigments

After the incubation period of 6 weeks, the mycelium was harvested, and the broth was filtered in a sterilized muslin cloth. Later, two volumes of 95% (v/v) ethanol was added to exhausted culture broth according to the following procedure: (i.) after dilution with about 60% of the solvent volume needed, the resulting mixture was
kept on the rotary shaker at 180 rpm at 30 °C for 30 min; (ii.) the ethanolic mixture was centrifuged at 4000 rpm for 15 min; (iii.) once the supernatant had been recovered, the residue was dispersed in the remaining volume of ethanol and centrifuged again at 4000 rpm for 5 min; and (iv.) the supernatants were then collected and filtered through a Whatman filter paper (47 mm) and further diluted with 95% (v/v) ethanol to a final volumetric dilution factor of 20. Next, the absorption spectrum was observed at 300–650 nm using JENWAY-6305 spectrometer (Santis et al., 2005). The purified pigments were concentrated in a buchi rotary evaporator and lyophilized to obtain powder (Velmurugan, et al., 2010). The optical density (OD) was measured at 250, 300, 350, 400, 450 and 500 nm (The wavelength which refers to the absorption maxima for red pigment).

4.3.6 Biomass estimation (Dry cell weight)

The fungal biomass in the synthetic medium was filtrated through a preweighed Whatman filter paper GF/C disc (47 mm) and washed twice with deionised water followed by drying at 105 °C for overnight. The dried samples were cooled to room temperature in a desiccator for 2 hrs and then weighed. Biomass was determined by gravimetric analysis. The biomass concentrations were calculated using the following formula.

\[
\text{Biomass (gL}^{-1} \text{)} = \frac{(W_2 - W_1)}{V}
\]

where, \( W_1 \)-weight of empty filter paper, \( W_2 \)-Weight of dried biomass on the filter paper and \( V \)-volume of culture broth in litre.
4.3.7 Antibacterial activity of pigments

Fungal pigments were screened against human pathogenic bacteria (both Gram +ve & -ve) to analyse the antibacterial property, viz., *Escheritia coli* (MTCC723), *Staphylococcus aureus* (MTCC3160), *Klebsiella pneumoniae* (MTCC 7028), *Pseudomonas aerogenosa* (MTCC 3541), *Streptococcus pyogenes* (MTCC1924), *Salmonella typhi* (MTCC734), *Bacillus subtilis* (MTCC) and *Clostridium perlingens* (MTCC). Inoculated loopful of pathogenic bacterial cultures into the test tube having 5 mL sterile Luria Bertani broth and incubated at 37 °C for 24 h. After the incubation transferred 100 µl of the inoculums of the test pathogen was spread on to Nutrient Agar plates and spread plate method was done with a sterile swab. A well of 5 mm diameter well was made in each corner of the plates equidistantly, using a sterile cork borer. Filter sterilized (0.25 µm pore size) aqueous extracts of fungal pigments with four different concentrations like 25, 50, 100 and 200 mg/ml compared with standard antibiotic chloromphenicol (10 mg/ml) were tested. 50 µl of each concentration of pigments and standard antibiotic was transferred into their respective labelled wells and the plates were incubated at 37 °C for 24-48 h. sterile water was used as a control. Each bacterial strain was tested against the fungal pigments with three replications during the study. After the incubation, the inhibition zone (minimal inhibitory concentration) around the well was recorded and expressed in millimeter (mm).

4.3.8 Antifungal activity of pigments

Fungal pigments were screened against human pathogenic and dermatophytic fungi to analyse the antifungal property viz., *Candida albicans*
(MTCC1637), Microsporum gypsium (MTCC2819), Chrysosporium keratinophilum (MTCC1367), Chrysosporium meridium (MTCC4608), Chrysosporium indicum (MTCC4965) and Trichophyton rubrum (MTCC3272). Pathogenic and dermatophytic fungal culture suspensions were prepared by transeferring 2 loopful of fungal spores into 5 mL of sterile distilled water with non-ionic detergent Tween 20 with a sterile inoculation loop and mixed homogenously. Transferred 100 µl suspensions of test pathogens on to the Potato Dextrose Agar plate and spread plate method was followed. A well of 5 mm diameter was made in each corner of the plate equidistantly using the sterile cork borer. Filter sterilized (0.25 µm pore size) aqueous extract of fungal pigments with four different concentrations like 25, 50, 100 and 200 mg/ml compared with standard antibiotic Fluconazole (50 mg/ml) was tested. 50 µl of each concentration of pigments and standard antibiotic was transferred into their respective labelled wells and the plates were incubated at 27 ± 2 °C for 72-120 h. and sterile water was used as a control. Each fungal strain was tested against the fungal pigments with three replications in this study. After the incubation, the inhibition zone (minimal inhibitory concentration) around the well was recorded and expressed in millimeter (mm).

4.3.9 UV and IR Spectral analysis of pigments

UV and IR spectra were done for the dried crude extracellular fungal pigments, absorption maxima (λmax) and characteristic peaks were identified and analyzed. UV absorption maxima peaks were measured in terms of nanometers (nm) and IR peaks were measured in terms of wavenumbers (cm⁻¹), (Lee and Kim, 2004).
4.3.10 Dyeing of textiles with extracted pigments

Fungal pigments were widely used as natural textile dyes instead of the synthetic dyes. Present study was followed for the dyeing of cotton and silk fabrics using four water soluble fungal pigments like orange red, yellow, red brown and red. Mordanting can be done by two methods like Pre-mordanting and post mordanting methods. Mordanting was done for the silk and cotton fabrics with different mordants like alum (Potassium Aluminum Sulphate) and ferrous sulphate (FeSO₄). Dyeing of cotton and silk fabrics were done with the different per cent of dye shades of four water soluble extracellular fungal pigments like 1, 2, 5 and 10% respectively, according to the method described by Lee and Kim, (2004).

Pretreatment

Degummed and bleached plain silk and cotton fabrics were purchased from Textile Company, near Davanagere. They were further treated with 5 g/L Non-ionic detergent (o.w.f) at a liquor ratio of 1:50, for one hour in boiling water, then thoroughly rinsed with hot and cold water sequentially and dried at ambient temperature.

Pre-mordanting and dyeing

Silk and cotton fabrics were pre-mordanted with different mordants like 5% of alum and 10% of FeSO₄ (o.w.f), conventionally at 40°C for 60 min. at a liquor ratio of 1:50, under boiling water bath shaker by maintaining pH of 4 for silk and 10 for cotton, each of separately in stainless steel jars, along with maintained control fabrics.
Followed by this, fabrics were rinsed with warm and cold waters to remove uncoated mordant and dried.

**Postmordanting**

Dyed and air dried silk and cotton fabrics were mordanted with 5% alum (o.w.f) and 10% FeSO₄ (o.w.f), conventionally at 40 °C for 60 min. at a liquor ratio of 1:50, under boiling water bath shaker by maintaining pH of 4 for silk and 10 for cotton, each of separately in stainless steel jars, along with maintained control fabrics. Followed by this, fabrics were rinsed with warm and cold waters to remove uncoated mordant and dried.

**Dyeing**

Extracted and completely moisture dried fungal pigments of orange red, yellow, red brown and red colours were used. Premordanted and pretreated fabrics were dyed separately with different per cent of shades (o.w.f) of extracted fungal pigments like 1, 2, 4, 5 and 10% (o.w.f). Premordanted and pretreated fabrics were used for dyeing at 60 °C for 60 min. at a liquor ratio of 1:50 (owf), under boiling water bath shaker, in comparison with the conventional method at 60 and 80 °C for 60 to 90 min. Dye bath pH of 4 for silk and 10 for cotton was maintained to control the dye uptake. The dyed silk and cotton samples were soaped for 30 min at 60°C, with 2 g/L Nonionic detergent, rinsed with warm and cold water to remove the uncoated dye and air dried (Mansour, 2010; Gorgani and Taylor, 2006). Pretreated and dyed cotton and silk fabrics were used for the post mordanting methods and premordanted and dyed silk and fabrics were ironed and used for the measurement of dyeing efficiency.
4.3.11 Measurements

Colour strength (K/S) on fabric Reflectance measurements on the dry dyed silk and cotton fabrics were carried out along with the control fabrics, using Cary 100 UV–Vis Spectrophotometer, giving reflectance values at wavelengths between 300 and 800 nm. From these values of reflectance Kubelka–Munk values (K/S) were calculated according to the equation (Kubelka, 1948).

\[
\frac{F}{R} = \frac{(1 - R)^2}{2R} = \frac{K}{S}
\]

where, K is the absorption coefficient, S is the scattering coefficient for a colourant at a specific wavelength, and R is the fractional reflectance value of the dye on the substrate at the \( \lambda_{\text{max}} \). The K/S value at \( \lambda_{\text{max}} \) is directly proportional to the concentration of dye on the substrate.
4.4 RESULTS

Screening, isolation and characterization of effective extracellular pigment producing fungi were done from the forest soils of Bhadra wildlife Sanctuary. The isolated fungal species like, *Paecilomyces farinosus*, *Emericella nidulans*, *Paecilomyces sinclairii* and *Penicillium purpurogenum*, they were producing orange red, yellow, red brown and red coloured pigments extracellularly.

4.4.1 Production of extracellular fungal pigments

Extracellular fungal pigment production was done from the fungi in a submerged state fermentation after the 45 days of incubation under static and dark condition. Ethanol extraction, filtration and drying process gives the little quantity of concentrated fungal pigments. Maximum yield of fungal pigment was produced by the *Peacilomyces sinclairii* (5.4 g/L), followed by *Penicillium purpurogenum* (2.3 g/L), *Peacilomyces farinosus* (1.8 g/L) and *Emericella nidulans* (1.5 g/L). Maximum dry cell biomass was yielded and recovered from the fungus *P. purpurogenum* (8 g/L), followed by *P. farinosus* (6.6 g/L), *P. sinclairii* (6.5 g/L) and *E. nidulans* (4.8 g/L). Change in the pH of the production medium was observed by the fungus after the completion of the incubation period of 45 days in the dark condition, which was changed the broth pH neutral to alkaline. More alkaline pH was observed in the culture broth of *P. sinclairii* (11.0), *P. purpurogenum* (10.0) and *P. farinosus* (9.5) and acidic pH was observed in the culture broth of *E. nidulans* (5.5) (Table. 4.1) (Plate. 9 & 10).
Table 4.1 Production of extracellular fungal pigments

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Species</th>
<th>Pigment Colour</th>
<th>Production Broth pH</th>
<th>Biomass (g/L)*</th>
<th>Yield of pigment (g/L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before incubation</td>
<td>After incubation*</td>
<td>Wet weight</td>
<td>Dry weight</td>
</tr>
<tr>
<td>1</td>
<td><em>Paecilomyces farinosus</em></td>
<td>Orange Red</td>
<td>7.0</td>
<td>9.5±0.1</td>
<td>45.8±1</td>
</tr>
<tr>
<td>2</td>
<td><em>Emericella nidulans</em></td>
<td>Yellow</td>
<td>7.0</td>
<td>5.5±0.25</td>
<td>19.6±1.2</td>
</tr>
<tr>
<td>3</td>
<td><em>Paecilomyces sinclairii</em></td>
<td>Red Brown</td>
<td>7.0</td>
<td>11±0.5</td>
<td>26±1.5</td>
</tr>
<tr>
<td>4</td>
<td><em>Penicillium purpurogenum</em></td>
<td>Red</td>
<td>7.0</td>
<td>10±0.45</td>
<td>28.8±1.8</td>
</tr>
</tbody>
</table>

*Mean of three replicates ± standard deviation (SD)
Plate 9

Isolation (a) and production (b) of extracellular fungal pigment

1. *Paecilomyces farinosus*
2. *Paecilomyces sinclairii*
3. *Emeriell nidulans*
4. *Penicillium purpurogenum*

Plate 10

Ethanolic extraction of fungal pigment

1. Orange red
2. Yellow
3. Red brown
4. Red
4.4.2 Antibacterial activity

Antibacterial activities of fungal pigments were done for Gram +ve and Gram –ve bacteria with four different concentrations of fungal pigments to analyse the minimum inhibitory concentration of the fungal pigments against human pathogenic bacteria.

Yellow coloured pigment was showed maximum growth inhibition towards the pathogenic bacteria like Clostridium perfringens, Streptococcus pyogenes, Pseudomonas auregenosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia and Salmonella typhi at the minimum inhibitory concentration of 100 mg/mL followed by 200 mg/mL, but other pigments showed maximum growth inhibition towards the tested pathogenic bacteria only at the minimum inhibitory concentration of 200 mg/mL by orange red, red brown and red coloured pigment respectively. Fungal pigments in comparison with the standard antibiotic Chloromphenicol with the concentration of 25 mg/mL, fungal pigments were showed less activity against the tested human pathogenic bacteria (Table 4.2).

4.4.3 Antifungal activity

Antifungal activities of fungal pigments were done for human pathogenic and dermatophytic fungi with four different concentrations of fungal pigments to analyse the minimum inhibitory concentration of the fungal pigments against human pathogenic and dermatophytic fungi.
Table. 4.2 Antibacterial activity of pigments

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Orange red (mg/mL)</th>
<th>Yellow (mg/mL)</th>
<th>Red brown (mg/mL)</th>
<th>Red (mg/mL)</th>
<th>Chloromphenicol (25 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>200</td>
<td>25</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Pseudomonas auregenosa</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>14</td>
<td>16</td>
</tr>
</tbody>
</table>

Table. 4.3 Antifungal activity of pigments

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Orange Red (mg/mL)</th>
<th>Yellow (mg/mL)</th>
<th>Red Brown (mg/mL)</th>
<th>Red (mg/mL)</th>
<th>Flucanazole 50 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>200</td>
<td>25</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Microsporum gypseum</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Chrysosporium keratinophilum</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Chrysosporium meridium</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Chrysosporium indicum</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>
Red coloured pigment was showed maximum growth inhibition at minimum inhibitory concentration of 25 mg/mL followed by 50, 100 and 200 mg/mL towards the Candida albicans and Chrysosporium keratinophilum, at 50 followed by, 100 and 200 mg/mL concentrations towards the Microsporum gypsium and Chrysosporium indicum, at 100 followed by, 200 mg/ml concentrations towards the Trychophyton rubrum and at 200 mg/mL concentration towards the Chrysosporium meridium. Yellow coloured pigment was showed maximum growth inhibition at the minimum inhibitory concentration of 25 mg/mL followed by, 50, 100 and 200 mg/mL towards the Chrysosporium keratinophilum, at 200 mg/mL concentration towards the Candida albicans, Chrysosporium indicum, Microsporum gypsium, Chrysosporium meridium and Trychophyton rubrum. Orange red coloured pigment was showed maximum growth inhibition at the minimum inhibitory concentration 25 mg/mL followed by, 50, 100 and 200 mg/mL towards the Chrysosporium keratinophilum, at 100 and 200 mg/mL concentrations towards the Trychophyton rubrum and at 200 mg/mL concentration towards the Microsporum gypsium, Chrysosporium indicum, Candida albicans and Chrysosporium meridium. Red coloured pigment was showed maximum growth inhibition at the minimum inhibitory concentration of 100 mg/mL followed by 200 mg/mL towards the Chrysosporium keratinophilum and at 200 mg/mL concentration towards the Candida albicans, Microsporum gypsium, Chrysosporium meridium, Trychophyton rubrum and Chrysosporium indicum. Fungal pigments in comparison with the standard antifungal antibiotic Fluconazol with the concentration of 50 mg/mL, fungal pigments were showed moderate activity against the tested human pathogenic and dermatophytic fungi (Table. 4.3).
4.4.4 UV and IR spectral analysis of fungal pigments

Analysis of UV-spectral results of four crude water soluble extracellular fungal pigments gives the maximum absorption ($\lambda_{\text{max}}$) peaks at the different nanometers. Pigments of orange red colour showed absorption maxima ($\lambda_{\text{max}}$) of 257, 332, 362 and 595 nanometres, yellow colour showed absorption maxima ($\lambda_{\text{max}}$) of 260, 414, 449, 598 and 825 nanometres, red brown colour shows absorption maxima ($\lambda_{\text{max}}$) of 257, 381 and 605 nanometres and red colour showed absorption maxima ($\lambda_{\text{max}}$) of 262, 319 and 467 nanometres (Fig. 4.1).

Analysis of IR-spectral results of four crude water soluble extracellular fungal pigments gives the peaks at the different wavenumbers (cm$^{-1}$). Orange red coloured pigment showed peaks at the wavenumbers of 3355 indicates the $-\text{OH}$ group, 1362 and 1574 indicates the $\text{C=C}$ stretching vibration, 1389 and 1334 indicates the $\text{C=C}$ stretching vibration and 1072 indicates the $-\text{C-O}$ groups (Fig. 4.2). Yellow coloured pigment showed peaks at the wavenumbers of 3322 indicates the $-\text{OH}$ group, 1635 indicates the $\text{C=C}$ group, 1396, 1305 and 1245 indicates the $\text{C=C}$ stretching vibration and 1074 indicates the $-\text{CO}$ group (Fig. 4.3). Red brown coloured pigment showed peaks at the wavenumbers of 3347 indicate the $-\text{OH}$ group, 1634 indicates the $\text{C=C}$ group and 1088 indicates the $-\text{CO}$ group (Fig. 4.4). Red coloured pigment showed peaks at the wavenumbers of 3331 indicates the $-\text{OH}$ group, 2136 indicates the $\text{C=\equiv C}$ group, 1634 indicates the $\text{C=C}$ group, 1395 indicates the $\text{C=C}$ group and 1077 indicates the $-\text{CO}$ group (Fig. 4.5).
Fig. 4.1 UV - Spectra of orange red (A), yellow (B), red brown (C) and red (D) coloured pigments
Fig. 4.2 IR - Spectra of orange red coloured pigment

Fig. 4.3 IR - Spectra of yellow coloured pigment
Fig. 4.4 IR - Spectra of red brown coloured pigment

Fig. 4.5 IR - Spectra of Red coloured pigment
4.4.5 Dyeing of textiles with extracted fungal pigments

Dyeing of cotton and silk fabrics were done with the four water soluble extracellular fungal pigments like orange red, yellow, red brown and red and mordants like alum and FeSO₄ using pre and post mordanting and dyeing methods.

Dyeing of silk and cotton fabrics was done with pre and post-mordanting technique using 5 and 10% of alum as a mordant and dyeing with 1, 2, 4, 5 and 10% of dye shades, they did not showed any effective dyeing using these methods.

Dyeing of silk and cotton fabrics was done with pre and post mordanting technique using 10% of FeSO₄ as a mordant and dyeing of four pigments with 1, 2, 4, 5 and 10% of dye shades. Postmordanting of 10% FeSO₄ and dyeing of four pigments with 1, 2, 4, 5 and 10% of dye shades and premordanting of 10% FeSO₄ and dyeing with 1, 2, 4, and 5% of dye shades did not showed any effective dyeing using these methods. But only premordanting of 10% FeSO₄ and dyeing of red pigment with 10% of dye shade showed effective dyeing than the others.

Dyeing of cotton fabric was showed maximum colour strength (K/S) in the wavelength range of 400-700 nm by the red pigment (1.86), followed by red brown (1.73), orange red (1.72) and yellow (1.7), when compared with the control (1.65) and mordanted (1.7) fabrics (Table. 4.4), (Plate. 11). Dyeing of silk fabric was showed maximum colour strength (K/S) in the wavelength range of 400-700 nm by the red pigment (3.8), followed by red brown (3.34), orange red (3.25) and yellow (3.2), when compared with the control (3.12) and mordanted (3.176) fabrics (Table. 4.5), (Plate. 11).
Table 4.4 Dyeing of Cotton with water soluble pigments

<table>
<thead>
<tr>
<th>Pigment</th>
<th>FeSO₄ (%)</th>
<th>Dye (%)</th>
<th>Liquor Ratio</th>
<th>K/S Ratio (400-700 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.65</td>
</tr>
<tr>
<td>Mordant</td>
<td>10</td>
<td>-</td>
<td>1:50</td>
<td>1.7</td>
</tr>
<tr>
<td>Orange Red</td>
<td>10</td>
<td>10</td>
<td>1:50</td>
<td>1.72</td>
</tr>
<tr>
<td>Yellow</td>
<td>10</td>
<td>10</td>
<td>1:50</td>
<td>1.7</td>
</tr>
<tr>
<td>Red Brown</td>
<td>10</td>
<td>10</td>
<td>1:50</td>
<td>1.73</td>
</tr>
<tr>
<td>Red</td>
<td>10</td>
<td>10</td>
<td>1:50</td>
<td>1.86</td>
</tr>
</tbody>
</table>

Table 4.5 Dyeing of Silk with water soluble pigments

<table>
<thead>
<tr>
<th>Pigment</th>
<th>FeSO₄ (%)</th>
<th>Dye (%)</th>
<th>Liquor Ratio</th>
<th>K/S Ratio (400-700 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.12</td>
</tr>
<tr>
<td>Mordant</td>
<td>10</td>
<td>-</td>
<td>1:50</td>
<td>3.176</td>
</tr>
<tr>
<td>Orange Red</td>
<td>10</td>
<td>10</td>
<td>1:50</td>
<td>3.25</td>
</tr>
<tr>
<td>Yellow</td>
<td>10</td>
<td>10</td>
<td>1:50</td>
<td>3.2</td>
</tr>
<tr>
<td>Red Brown</td>
<td>10</td>
<td>10</td>
<td>1:50</td>
<td>3.34</td>
</tr>
<tr>
<td>Red</td>
<td>10</td>
<td>10</td>
<td>1:50</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Plate 11

Dyeing of cotton and silk fabrics with fungal pigments
(Orange red, Yellow, Red brown and Red)

Pretreatment

Premordanting

Dyeing

Incubation

Control Test

Control Test

Dyed silk fabric

Dyed cotton fabric
4.5 DISCUSSION

The present study shows an idea about the screening and isolation of the extracellular pigment producing fungi and their diversity in the forest soil samples. In the present study, an attempt has been made to identify species of pigment producing fungi and to make use of the colour for textile and to analyse antimicrobial property and also we can use these pigments as food colours. Four water soluble pigments producing fungal species like \textit{Paecilomyces farinosus}, \textit{Emericella nidulans}, \textit{Paecilomyces sinclairii} and \textit{Penicillium purpurogenum}, these isolates were secreted very intense colours of orange red, yellow, red brown and red pigments respectively. As these four isolates consistently registered very high quantity of pigment secretion, they were selected for detailed studies.

Microorganisms occupy a distinct place as source of natural colours. More than bacteria, fungi display a wide range of fascinating colours, however, till recently fungi are remained unexplored for colour production. This is perhaps due to their association with mycotoxins and aflatoxins. The current trend in society for natural ingredients has stimulated interest in exploring novel means and sources for the biotechnological production of colourants. In this regard, exploring fungal chemical diversity is a worthwhile route for the identification of novel pigments. An intelligent screening approach for water-soluble pigments that is partly based on chemotaxonomy, it will provide a platform for the future construction of cell factories to the production of natural colourants. If imperative toxicological testing is carried out, fungal pigments could be accepted by the current consumer as a food and textile colourant. Further research using new technologies suggests a promising future for this field of study. Fungal pigments are widely using as food colours as well as in textile and
pharmaceutical industries, because these pigments having antimicrobial, antioxidant, anticancerous properties.

The present study the production of four water soluble pigments was done from the selected fungal species. Maximum pigment yield was produced by *Paecilomyces sinclairii* than the other species and maximum dry biomass was produced by *Penicillium perpurogenum* than the other isolates. The pH of the fermenting broth could influence the pigment production and biomass yield and the quality of the pigment (Morton and Macmilliam, 1954). Chen and Johns (1993) explored that fungal growth and ankaflavin synthesis were favoured at low pH where as the production of other pigments was relatively dependent of pH.

Our study showed higher biomass and pigment production at 25 ± 2 °C under dark condition, however similar results are reported by Wong et al., (1981). Many scientists working with *Monascus* spp., they reported that, 35 °C is an optimum temperature for the pigment production (Border and Koehler, 1980; Wong and Koehler, 1981). In addition to the effect of temperature, denaturation of the cell membranes might also occur at higher temperatures, which affect the extraction process (Cacace and Mazza, 2003). The pigments are heat-stable and hence, temperature of the extracting medium need not be increased to maximize the process. Cacace and Mazza, (2003) reported that, the higher temperatures may leads to the degradation of pigments.

Velmurugan et al., (2010) studied that, the effects of different wavelength of light on the biomass production at the weight of the dry biomass after 7 days of incubation. The biomass recovered from total darkness showed profuse growth giving a
fluffy appearance to the colonies and highly pigmented mycelia for *M. purpureus* and *P. purpurogenum*, followed by red and blue light source obtained maximum biomass weight in *M. purpureus* and *P. purpurogenum* and flasks exposed to white unscreened, green and yellow light showed the moderate biomass production in *I. farinosa*, *E. nidulans* and *F. verticillioides*.

*Paecilomyces sinclairii*, belongs to the class ascomycetes, has a capability of producing red pigment in high yields during submerged cultures (Miyake *et al.*, 1982). Generally, two growth forms, the filamentous and the pelleted forms can be observed in most fungal fermentations, in which the pelleted form is usually less viscous than the filamentous form (Berovic *et al.*, 1991). Although fungal morphology is influenced by a variety of factors such as medium composition, agitation intensity, and oxygen tension, it is still difficult to relate the broth rheology to every aspect of microbial morphology (Suijdam and Metz, 1981; Schugerl *et al.*, 1983; Goudar *et al.*, 1999; Cho *et al.*, 2002).

Although traditional processes in Asia have predominantly employed solid state fermentation methods, but liquid state fermentation or submerged fermentation is the classical method still in use in western countries. Lin (1973) demonstrated that *Monascus* spp. could be cultivated as submerged culture and pigment yields could be improved. Yamaguchi *et al.* (1973) developed a method to harvest water soluble pigments in submerged fermentation by forming amino acids with protein as nitrogen source. This would cause rearrangement bases with the pigment skeleton which produced hygroscopic pigments. Lee *et al.* (2008) demonstrated that when incubation time increased, pigment production was also increased.
The present study of four water soluble fungal pigments as antimicrobial agents, they were effective against human pathogenic bacteria and fungi, which showed good activity at the lower concentrations. Antibacterial activity of fungal yellow coloured pigment was showed maximum growth inhibition towards the both gram –ve and gram +ve pathogenic bacteria at minimum inhibitory concentration of 100 mg/mL, but other pigments like orange red red brown and red pigments at 200 mg/mL concentrations towards the tested pathogenic bacteria.

Antifungal activity of fungal red coloured pigment was showed maximum growth inhibition towards the Candida albicans and Chrysosporium keratinophilum fungal species at the minimum inhibitory concentration of 25 mg/mL, at 50 mg/mL towards the Candida albicans and Chrysosporium keratinophilum, at 100 mg/mL towards the Trychophyton rubrum and at the 200 mg/mL towards the Chrysosporium meridium. Yellow coloured pigment was showed maximum growth inhibition at the minimum inhibitory concentration of 25 mg/mL towards the Chrysosporium keratinophilum and 200 mg/mL concentration towards the remaining tested pathogenic fungal species. Orange red coloured pigment was showed maximum growth inhibition at the minimum inhibitory concentration 25 mg/mL towards the Chrysosporium keratinophilum, at 100 mg/mL concentration towards the Trychophyton rubrum and at 200 mg/mL concentration towards the other tested pathogenic fungi. Red coloured pigment was showed maximum growth inhibition at the minimum inhibitory concentration of 100 mg/mL towards the Chrysosporium keratinophilum and at 200 mg/mL concentration towards the other tested pathogenic fungal species. These results
reveal that the antimicrobial property of fungal pigments may be by the anthraquinone compounds or their derivatives.

A series of preliminary dyeing tests on different pre-mordanted cotton and silk fabric standard specimens using ethanolic extracts containing prevailing orange red, yellow, red brown and red pigments from *P. farinosus*, *E. nidulans*, *P. Sinclairii* and *P. pupurogenum* gave rise to dyed specimens with different hues depending on the mordant used. The colourants used for dyeing purpose worked at pH 4 for silk and 10 for cotton in the absence of glabours salt. Dyeing without salt addition was advantage and it was similar to those reported by Kamel et al., (2005). According to Lee and Kim, (2004) the K/S value of dyed cotton fabrics was increased with increasing the pH of buffer solution up to about 9, and thereafter it levelled off. The K/S value of dyed silk fabrics was not depended on the pH of buffer solution. However, the pH 9 gave the highest K/S value for cotton and silk fabrics. The reason of the different effect of pH on K/S for cotton and silk fabrics is not clear at the present moment. More detailed study is needed.

Ali et al., (2009) studies reported the effect of pre-mordanting and post-mordanting with aluminium sulphate and ferrous sulphate under different and reported that pre-mordanting and post-mordanting with ferrous sulfate, there was huge change in hue and a great deal of decrease in the chroma or purity of colour. Lee and Kim, (2004) reported in their study that, the K/S values of pre-mordanted cotton and silk fabrics were higher than those of un-mordanted fabrics. Generally, it is known that the K/S values of cotton fabrics dyed with natural dyes are very low. However, in his study, he also shows that premordanted and un-mordanted cotton fabrics dyed with *Cassia tora* L. extract were high K/S values. This result might be attributed to the higher affinity of anthraquinone dye with cellulose. The K/S values of silk fabrics were higher than that of cotton fabrics. This might be due to the higher mordanted metal ion content.
4.6 Summary

The primary objective of this study is to isolate and select the pigment producing fungi for the application aspect studies, then explore the fungal pigments as a sources for analysing antimicrobial compounds and dyeing colourants. Owing to the conflicting reports on the safety of food and textiles during processing and dyeing, due to the indiscriminate use of non-permitted colours, there is an urgent need to identify natural pigment sources as safe food and textile colourants. This study critically examines the potentials of fungi as sources for production of biocolourants.

Soil samples collected from Bhadra Wildlife Sanctuary, they are rich sources of diversified and efficient fungal species for the production of industrially important products. Four water soluble pigment producing fungi were isolated and characterized. Potent pigment producing fungal isolates such as Paecilomyces farinosus, Emericella nidulans, Paecilomyces sinclairii and Penicillium purpurogenum, they are having the capable of producing orange red, Yellow, red brown and red coloured pigments extracellularly, they were studied and used for production technology. Antimicrobial and dyeing tests were done with four water soluble pigments like orange red, yellow, red brown and red in different concentrations and evaluated. In the present study medium pH 7.0, temperature of 25 ± 2°C, dark and static condition was used for the production of pigments from the selected species. Maximum pigment yield was produced by Paecilomyces sinclairii and maximum dry biomass was produced by Penicillium purpurogenum than the other isolates.
In the present study of antibacterial activity of yellow coloured fungal pigment showed maximum growth inhibition at the minimum inhibitory concentration 100 mg/mL towards all tested pathogenic bacteria. Antifungal activity of red coloured fungal pigment was showed maximum growth inhibition at the minimum inhibitory concentration of 25 mg/mL towards the Candida albicans and Chrysosporium keratinophilum, at 50 mg/mL concentrations towards the Microsporum gypseum and Chrysosporium indicum than the other tested pathogens.

Dyeing of cotton fabric was showed maximum colour strength (K/S) in the wavelength range of 400-700 nm by the red pigment, followed by red brown, orange red and yellow when compared with the control and mordanted fabrics. Dyeing of silk fabric was showed maximum colour strength (K/S) in the wavelength range of 400-700 nm by the red pigment followed by, red brown, orange red and yellow, when compared with the control and mordanted fabrics.