CHAPTER-2
REVIEW OF LITERATURE

Pigments play an important role in every step of our lives, because pigments are present in all the organisms, where plants and microorganisms are the principal colour producers. Both natural and synthetic pigments were used in many industries like textile, furniture, cosmetics, medicines, foods, clothes, and in many other products. Natural pigments have so many important functions, such as photosynthesis by chlorophylls and carotenoids. Pigments have a well-known pharmacological activity such as antioxidant and anticancer (Cristea and Vilarem, 2006; Delgado-Vargas et al., 2000)

Pigments produced from natural sources are generated interest worldwide and is gaining importance and the demand to produce natural sources of colour is increasing day by day because of the positive health related benefits out of natural compounds (Cristea and Vilarem, 2006). Therefore, it is necessary to research on various natural sources of colorants and their potentials (Delgado-Vargas, et al., 2000). Ores, insects, plants and microbes are many other source of natural colorants which play a remarkable role as food coloring agent, because of its production and easy down streaming process (Bechtold and Mussak 2009; Delgado-Vargas and Paredes-Lopez, 2002).

2.1 History of colorants

In 1856, H. Perkin established the first industrial unit of organic synthetic dyes to produce purple colour. A few years later the discovery of diazotization and a coupling reaction by Peter Griess was the next major advance for development of the colour industry (Sikorski, 2006). Synthetic organic dyes were developed till end of 19th century, which was more inexpensive and wider range of colorants. Intensive research and developments enhanced the quality of colorants. The economic significance of the colour industry is clearly reflected in the large number of synthesized compounds; as many as 700 colorants are currently available (Delgado-Vargas and Paredes-Lopez, 2002).
Toward the end of the 19th century, when synthetic colours were first adopted for use on a large scale, they were hailed as a considerable technological breakthrough. The term 'synthetic' was associated with the idea of progress and synthetic colorants were actually considered safer in food than the naturals, as they were tinctorially much stronger and consequently a smaller quantity was needed to achieve a specific colored effect (Sikorski, 2006). Many synthetic colorants were used in many areas like, but through the years their importance reduced. This cutback of synthetic colorants started about five decades ago. All synthetic food components have reported severe criticism, including synthetic food additives and mostly food pigments.

Colour additives were one of the first man-made (synthetic) products regulated by law. Today, all food colour additives are cautiously regulated by federal authorities to ensure that foods are safe to eat and accurately labeled (Freedman, 1978). Colour plays an important role as an important marker of food quality. The consumer links food color with good processing and safety (Neitz et al., 2002).

Primarily coloring agents can be divided into two classes i.e. pigments and dyes. Dyes are water soluble substances and have at least one salt-forming group (Satake and Mido, 1995). Pigments are the particulate solids disperse into a medium without significant solution or their interactions. They are oil-soluble or solvent-soluble colorants lack with salt-forming groups. These pigments occupy a major place in our daily life. The most primitive known pigments were natural minerals. Natural iron oxides, anhydrous ferric oxide, charcoal and so on are several well-known pigments since prehistoric times (Stafsnes, 2010).

2.2 Microbial pigments

Natural pigments are available in huge number but very few are available in adequate quantities for large scale production. The microorganisms can grow rapidly and may lead to a high productivity (Venil et al. 2013; Dufosse et al., 2005).

2.2.1 Fungal pigments

Fungi also produces pigment during their growth and specified by permanent staining which is most often associated with growth of textile and plastics. Pigments
produced by fungi also have ability to produce production of dyes to replace synthetic pigments. Microbial pigments are explored as colorants in textile industries (Hobson and Wales, 1998).

2.2.2 Bacterial pigments

Some bacteria produce pigments which can be observed after they grow into colonies. Pigments can aid to identify bacteria. Water soluble pigments which are produced by some bacteria spread through the medium in which they grow and many others are soluble in fat (Soliev et al., 2011). Flavobacterium sp. produces yellow pigment zeaxanthin, which is used as an additive in poultry feed tofortify the yellow color of skin of birds. Zeaxanthin is also used in cosmetic and food industries. In fish feed canthaxanthin from bacterium Bradyrhizobium sp. has been used for many years (Dufosse et al., 2005).

2.2.3 Yeast pigments

Yeast produces some valuable carotenoids in culture on comparative low cost substrates, hence they are providing very good alternative to chemically synthesized pigments. Asthaxanthin from Xanthophyllomyces dendrohous formerly known as Phaffia rhodozyma is a carotenoid pigment which is used as a food colorant and widely used in the animal feed to impart color to the animal skin as animals have no capacity to synthesis the carotenoid pigment.

Hundreds of scientific papers and patents deal with asthaxanthin production using this yeast and pigment production process has not been economically efficient till now. Most of the research in recent years is also focused on Rhodotorula glutinis which gives carotenoid pigment. However some papers reported reasonable pigment production with other species such as R. gracilis, R. rubra (now R. mucilaginosa) and R. graminis too. The main compounds obtained by these red yeasts are torulene and torularhodin with minute quantity of beta carotene (Dufosse, 2005).

The study of natural pigment is one of the emerging field of research. The researcher are now interested in studies of production and applications on natural pigments (Venil and Lakshmanaperumalsamy, 2009).
2.3 Isolation, screening & characterization of pigment producing actinomycetes

2.3.1 Actinomycetes

Actinomycetes are very important Gram positive, sporulating, filamentous, bacteria. Actinomycetes have DNA rich in G+C content ranging from 55-75% (Ho et al., 2002). Out of thousands of bioactive secondary metabolites produced by bacteria, actinomycetes are responsible for the production of more than fifty percent of antibiotics production (Berdy, 2005; Strohl, 2004), immunosuppressive agents production (Mann, 2001), antitumor agents production (Cragg et al., 2011), and various important enzymes production (Oldfield et al., 1998). Actinomycetes are not only rich source of bioactive metabolites; they are also rich source of effective biocontrol agents against phytopathogenic fungi (Yuan and Crawford 1995).

Palanichamy et al., (2011) reported isolation of actinomycetes from rhizosphere soil of different fields nearby VIT and coastal regions of Chennai using sterile containers and were brought to laboratory. The triplicates from each location were pooled together to increase the bacterial flora, which showed pigment producing ability along with antimicrobial activity.

The collected soil samples were serially diluted using sterile water and plated on Kenknight & Munaier's Medium, YMG agar and starch casein agar media using $10^{-3}$, $10^{-4}$ and $10^{-5}$ dilutions by spread plate and pour plate techniques. They were incubated for 5-7 days at 28-30°C. The pure colonies were isolated and again plated on different growth media including Kenknight & Munaier's Medium and Starch Casein Agar medium by streak plate method. The medium preparation for microorganisms to be isolated from marine sediment was done using 50% sea water.

Rhizosphere soils represent a unique biological niche with a diverse micro flora comprised of bacteria, fungi, actinomycetes, protozoa and algae. These microorganisms are very much dependant on organic materials nutritionally which are derived from plant roots and root exudates (Lynch, 1990). The root exudates quality and composition varies and depends on different plant species and environment such as humidity and temperature.
Ashokvardhan et al., (2014) reported that rhizosphere soil samples of Capsicum plants, collected from the agricultural fields located at various districts of Andhra Pradesh, India. The samples were taken from the growing roots up to a depth of 5 cm after removing approximately 3 cm of the soil surface. All samples were collected in pre-sterilized polythene bags, and stored for further analysis.

Isolation of actinomycetes from soil is relatively difficult as numerous bacteria and fungi interfere with the growing actinomycetes cultures. Several pre-treatment methods are proposed to avoid this difficulty.

Agate and Bhat, (1963) made an attempt to inhibit the contamination of bacteria and fungi by pre-heating the soil samples at high temperature (110°C) while treatment at 40°C for 2-16 h was suggested by Williams and Cross (1971). According to the research of Nolan and Cross (1988), the isolation and purification of rare actinomycetes was stimulated by drying and heating treatment, and they found rare actinomycetes by these treatments (Alferova et al., 1989).

Morphological characteristics of actinomycetes are employed in classification of actinomycetes. Genus Streptomyces mainly classified on the basis of spore chains and divided into three sections namely Rectiflexibles (RF), Retinaculiaperti (RA) and Spirales (S) (Shirling & Gottlieb, 1966).

The morphological, physiological, and biochemical characters are mainly used for classification of actinomycetes. The classical approach of identification of actinomycetes was described by two main sources. Nonomura (1974) and Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974). These methods are very much useful in the identification of Streptomyces. Lango et al., (1999), described Streptomyces galbus based on morphological and physiological traits (Arifuzzaman et al., 2010).

Amsaveni et al., (2015) collected soil samples from different locations like forest soil, river sediments and well soil in and around Coimbatore region, Tamil Nadu, India. The samples were collected and serially diluted up to $10^{-8}$ dilutions were done for all the soil samples and 100 μL from each dilution was spread plated on to Kuster's agar (Himedia). The culture plates were incubated at
37°C for 7 days and morphologically observed for actinomycetes colonies. The morphologically diffusible pigment producer colonies were selected and purified.

Hamaki et al., (2005) and Galatenko et al., (1982) reported that soil samples from different location was collected and processed by serial dilution method on different media. They observed the actinomycetes by different coloration of substrate and aerial mycelium and diffusible pigment producing ability and selected seven strains of actinomycetes. The strains were identified morphologically by light microscopy. They also performed gram’s staining to determine actinomycetes. Singh et al., (2012) also reported the isolates of the actinomycetes on the basis of biochemical studies. Various biochemical tests (Catalase test, Casein hydrolysis, Starch hydrolysis, Indole test, Triple Sugar Iron (TSI) agar) were performed for the identification of the isolates.

The determination of antimicrobial properties of actinomycetes showed a broad spectrum against microorganisms including bacteria, fungi, molds and dermatophytes. The antimicrobial properties are studied by Hayakawa et al. (2004), Oskay et al. (2004) and Dhanashekaran et al. (2005) using cross streak method and agar overlay method. Generally test organism B. subtilis, S. aureus, P. vulgaris, P. aeruginosa and B. cereus and S. aurius sp. C. albicans and A. niger were inhibited by isolates in previous studies.

Naidenova and Vladimirova, (2002); Augustine et al., (2004) reported that actinomycetes showed tremendous potential as producers of novel products with important bioactivities like bactericidal, fungicidal, antiviral and antitumor. Culture extracts of promising isolates of actinomycetes was screened for antimicrobial activity in secondary screening procedures by well agar diffusion method. The potency of the isolate was determined by zone of inhibition against test microbes.

2.4 Optimization of growth parameters for actinomycetes

Armen et al., (2004) identified the actinomycetes on the basis of different biochemical test like production of H₂S, starch hydrolysis, casein hydrolysis and gelatin hydrolysis, catalase and nitrate reduction. They also tested the effect of various physiological factors like pH, temperature and sodium chloride on the growth of selected actinomycetes.
Similar study was also carried out by Dastager et al., (2006) in which they studied various physiological growth factors on actinomycetes. Various carbon sources as glycerol, starch, dextrin, lactose, sucrose, fructose or glucose and nitrogen sources as L-asparagine, L-arginine, L-citrulline, L-histidine, glycine, L-lysine, L-proline or L-tyrosine was also assessed on the pigment production in *Streptomyces*.

Ali et al., (2011) showed the effect of various physiological conditions on actinomycetes. They revealed the effect of pH and incubation period on pigment production, effect of carbon sources supplemented with the utilized medium on pigment formation. Different concentrations of peptone were studied on the pigment formation. Effect of different temperature was also checked in their research.

Mohanshrinivasan et al, (2013) also reported their finding related to optimization of medium by proving different physiological sources. They studied pigment production and growth of actinomycetes by providing different pH, temperature, carbon sources and nitrogen sources.

### 2.5 Extraction and purification of pigment

Song et al., (2006) showed the method of solvent extraction in which the equal volume of petroleum ether was added to the methanol extract taken in a separatory funnel and mixed. Pigment was separated by silica gel column chromatography with solvent mixture of n-hexane and ethyl alcohol. Krishna et al., (2008), used different solvents such as acetone, methanol, petroleum ether, ethyl acetate, chloroform, ethanol, hexane distilled water and diethyl ether, for extraction of pigment.

Manjula et al., (2009) described the fermentation process in which the actinomycetes were cultured in a jar fermented with various sugar sources with or without sodium alginate beads.

Diraviyam et al., (2011) showed that the pigments were separated using readymade silica gel coated on alumina sheets (Merck). Separation was done using various organic solvents such as chloroform, methanol, acetic acid, water, ethyl acetate, dichloromethane and n-butanol in different proportions. After the
development of chromatogram, separated pigment was observed under UV lamp and also using iodine chamber.

Riguera (1997) described biomass extraction with an adequate solvent system (usually methanol or acetone), the isolation of a natural compound from the main extract or broth usually consists of a sequential gradient partition with solvents (Petroleum ether, hexane, carbon tetrachloride, chloroform, methylene chloride, ethyl acetate, n-butanol and water).

Vasanthabharathi et al. (2011) and Mohanshrinivasan et al., (2013) showed the fermentation in bioreactor and production of the pigment was carried out in an in-house developed bioreactor. The Gause's Medium with pH 7.8 was used.

Preeti et al., (2013) showed separation of crude pigment using various organic solvents such as chloroform, methanol, acetic acid and n-hexane in different proportions. After the development of chromatogram, separated pigment was observed using UV lamp and iodine chamber.

Atta (2011) carried out the process of fermentation for five days using starch nitrate as a medium and total volume filtered was conducted followed by centrifugation at 5000 rpm for 20 minutes and temperature was set to 10ºC. n-butanol and water (1:1 v/v) used for extraction of compound and used evaporator under reduced pressure and collected the crude compound. They also used the solvent system chloroform and methanol (24:1, v/v) to separate the antimicrobial compound.

Lalitha et al., (2011) reported that the ethyl acetate and n-butanol extract of the culture supernatant also showed significant results against various microorganisms. The fraction collected by Thin Layer Chromatography (TLC) technique and checked for their antifungal activity.

Zhang et al., (2005) used the whole culture broth of actinomycetes and added a solvent methanol and incubated at room temperature in a shaker. The mixture of broth and solvent were filtered and evaporated to dryness by using dryer incubator and then stored at 4ºC for further analysis. The culture filtrates and crude extracts were collected from the whole culture broth of the selected strains and were tested
antifungal activity against *F. oxysporum* and *A. alternata*. Extract of selected actinomycetes were evaluated for its antifungal activity by the modified agar well diffusion method.

Pandey *et al.*, (2011) used chloroform and methanol to extract bioactive compound. Hewedy and Ashour (2009) reported similar results in their experiment using different media as a production media for extraction of compound and mixed with equal amount of ethyl acetate. The extracted compound was dried by using heating mantle.

Selvameenal *et al.* (2009) collected cell free supernatant and mixed with equal amount of ethyl acetate (1:1). After evaporation, the crude compound was dissolved in distilled water and separated by thin layer chromatography using G-60 grade absorbent by solvent system chloroform: methanol: water (25:24:20). The Rf value was recorded. The TLC plates were exposed to iodine vapors and bands were collected separately and concentrated by methanol.

Jadon *et al.*, (2014) used starch casein nitrate broth for production of bioactive compounds from actinomycetes. Solvent extraction is generally used for the extraction of bioactive compounds from culture filtrate obtained from media and ethyl acetate was used to extract the bioactive compound because ethyl acetate is a good solvent due to its high polarity and high volatility.

### 2.6 Characterization of extracted compound

Kavitha and Vijaylaxmi, (2009) collected the active principle of the first fraction after HPLC purification. They analyzed the structure of bioactive compound by using various techniques like FTIR, GCMS, $^1$H NMR and $^{13}$C NMR.

Khanna *et al.*, (2011) used the following molecular techniques for tentative characterization of the isolates like TLC and UV-visible spectroscopy. Islam *et al.*, (2012) purified antibiotic compounds from isolated strain and used ethyl acetate for purification of antibiotic substances. $^1$H and $^{13}$C NMR spectral analysis was performed for identification of compound(s).

Nandani *et al.*, (2013) also published in their work that compound extracted from actinomycetes was purified and separated by thin layer chromatography and
further purified by column chromatography. A single spot on TLC plate confirmed the purity of compound and the Rf value of active compound was obtained. Mass spectrum analysis confirmed the identification of compound.

Usha et al., (2011); Mohanasrinivasan et al., (2013) and Kumar et al., (2011) also extracted and separated the compound by using thin layer chromatography and obtained Rf value. The characteristic three peak spectrum was detected in partially purified metabolite by UV–Vis spectrophotometer between 299.6 and 500 nm, which identified the lambda max of compound.

Janardhan et al., (2014) reported that the crude compound produced by the actinomycetes was purified by thin layer chromatography and the desired fraction collected from column chromatography was studied by HPLC for further purification and later examined by FTIR, NMR and Mass spectroscopy to obtain chemical structure. The compound also tested by In-vitro antioxidant assay and found nearby the standard ascorbic acid.

According to Saraswathi and Gricilda, (2015) TLC of the crude compound in order to identify the bioactive compounds on pre-coated silica sheet using capillary tube using Ethyl acetate: Toluene, in which the separation of compound were found with Rf values under UV and iodine illumination.

Taidala et al., (2016) reported the structural details using NMR, Mass spectroscopic and HPLC analysis. The extracted bioactive compounds were further analyzed by Mass and NMR spectroscopy for their molecular characterization.

Shaaban et al., (2013) showed the extracted pigment was insoluble in hydrochloric acid and organic solvents, (acetone, chloroform and ethanol) or mixtures of these solvents. They also used FT-IR and TLC for characterization of pigments.

2.7 Biological activity of pigment

2.7.1 Toxicity test and antioxidant properties

Suthindhira and Kannabiran., (2009) reported the cytotoxicity of bioactive compound, extracted from actinomycetes on HeLa cells by MTT assay and
compound showed cytotoxicity against both cell lines. The compound also showed a significant antimicrobial activity against various human pathogens which confirmed that bioactive compound has good antimicrobial and cytotoxicity.

Zarina and Nanda (2014) also tested the antioxidant property of crude extract of *Streptomyces* MS-60 by 1, 1-Diphenyl-pircrylhydrazyl (DPPH) assay and observed the scavenging activity. They also performed the MTT assay by using HT-29 (Human colon cancer cell) line and recorded IC$_{50}$ value.

Sudha and Selvam, (2013) also performed the MTT cytotoxicity assay and found IC$_{50}$ value against Hep-2 and VERO cell lines of crude compound extracted from actinomycetes. Similarly Selvam et al., (2013) reported an experiment of MTT assay against Gastric stomach cancer (AGS) cell line. L-asparaginase inhibited the AGS cells. The enzyme showed a good scavenging activity against hydrogen peroxide and 2, 2 - diphenyl - 1 - picrylhydrazyl (DPPH).

Rajan et al., (2012) also extracted the secondary metabolites from actinomycetes for antioxidant and cytotoxicity tests. The antioxidant assay was performed by DPPH method and scavenging activity was obtained. The cytotoxicity test was also performed by MTT assay against HeLa (human cervical cancer) cell lines and IC$_{50}$ value was found.

Krishna et al., (2014) also isolated *Streptomyces* sp. VITSJK8. The pure compound was extracted and further proceeds for cytotoxicity test by MTT assay against various cell lines such as mouse embryonic fibroblast (NIH 3T3) and human keratinocyte (HaCaT) normal cell lines, human hepatocellular liver carcinoma (HepG 2) and human breast adenocarcinoma (MCF-7) cell lines.

The cytotoxicity test of compound extracted from actinomycetes was also performed by Rashad et al., (2015). Cytotoxic activity against human heptaocellular carcinoma cell lines (HePG-2), human Caucasian breast adenocarcinoma (MCF 7) cell lines, Lung carcinoma cell lines (A-549) and Colon cell lines (HCT-116) was tested. They concluded that isolated strains of actinomycetes showed a broad spectrum of cytotoxicity towards most tested antitumor cell lines.
Naine *et al.*, (2011) tested the biological activity of crude extract of *Streptomyces parvulus* VITJS11 strain and performed DPPH assay to test antioxidant activity of extracted compound from strain. Aftab *et al.*, (2015) reported *Streptomyces* sp. KML-2 from saline soil. The strain showed cytotoxic activities against HeLa, MDBK and Vero cell lines.

Prashanthi *et al.*, (2015) reported cytotoxicity of bioactive pigment isolated from *Streptomyces* strain M045. They performed MTT assay with HeLa, Hep G3 and human lymphocytes cell lines. They took different concentrations of the pigment from 2.5, 5, 10 and 20 μg/mL.

Kumar and Krishnan, (2012) also researched DPPH radical scavenging and total antioxidant activity of DMBPO was compared with ascorbic acid as control and is given in Fig. 5. The radical scavenging activity of DMBPO was concentration dependent and gradual increase of concentration increased the activity.

Saraswathi and Gricilda, (2015) and Chakraborty *et al.*, (2015) reported antioxidant property of pigment, extracted from actinomycetes. They reported that Oxidative stress occurs in every cell due to metabolic processes. Environmental factors such as UV-radiation, ozone or smoke can induce oxidative stress. Antioxidant potential is the capability of a substance to scavenge free radicals available in its surroundings. DPPH (2,2-diphenyl-1-picryl-hydrazyl) is reported as a light sensitive crystalline colored compound which contains stable free radicals. Mani *et al.*, (2015), also reported the antioxidant activity of pigment from actinomycetes and showed scavenging activity.

### 2.7.2 Allergy test of pigmented compound

Ahn *et al.*, (2010) reported that from past two years some serious efforts has been generated to develop several In-vitro tests for eye and skin irritation in response of drug or test compound applications. The 3T3-Neutral red uptake assay is efficient and cost effective method as an alternative of In-vivo methods.

The neutral red uptake or NRU assay works on the ability of viable cells to incorporate and bind with supravital dye neutral red in the lysosome. It provides the quantitative data estimation of the number of viable cells in a culture. This assay
was included as part of the COLIPA international validation trial of \textit{in vitro} alternatives to the allergy test. \textcite{Yang2007} reported 3T3-NRU assay is a short-term test system based on a monolayer culture of 3T3 cells with cytotoxicity used as the endpoint for irritancy evaluation of test substances.

The present study tested and classified 23 cosmetic ingredients into three severity ratings, i.e. non-/mildly irritating, moderately irritating and strongly irritating, and the results were compared with those of Draize test. \textcite{Jirova2007} reported NRU assay as a replacement of \textit{In Vivo} study of compounds.

### 2.7.3 Enzymatic and non enzymatic activities

\textcite{Lekshmi2014} isolated actinomycetes and tested for enzymatic activities. The isolates were tested for four hydrolytic enzymes amylase, cellulose, protease and L-asparaginase. All four isolates were grown on starch agar media and allowed for incubation for 5 days. The amylase production was determined by the plates floated with iodine solution and appearance of clear zone around actinomycetes. The result indicated positive result. Out of four isolate, three isolates were shown positive result. The isolates were also showed cellulose, protease activities.

\textcite{Shah2016} also performed an experiment in which they tested actinomycetes isolated for their enzyme production ability. They reported that the actinomycets are potential source of hydrolytic enzymes. They produced cellulose L- asparaginase enzymes which have many industrial importances.

\textcite{Sonya2015} explored three \textit{Streptomyces} which have ability to produce the hydrolytic enzymes. All three isolates were incubated on starch casein agar medium and showed a clear zone around the culture. All three isolates found positive and produced cellulase, lipase and gelatinase.

### 2.7.4 Antimicrobial activity of pigment

\textcite{Maataoui2014} reported the antimicrobial properties of bioactive compound against various microorganisms like \textit{Mycobacterium aurum}, \textit{Bacillus subtilis}, \textit{Bacillus cereus}, \textit{Pseudomonas aeruginosa}, \textit{P. aeruginosa}, \textit{Candida albicans} and \textit{Candida tropicalis}. 
Selvameenal et al., (2009) mentioned in their research about antimicrobial property of pigment, isolated from desert soil. The crude pigment was screened for biological activity against drug resistant pathogens MRSA, VRSA and ESBL cultures of E. coli, Klebsiella sp., Pseudomonas aeruginosa by disc diffusion method.

Mani et al., (2015) also reported antimicrobial activity of pigment and resulted significant ability against various human pathogenic microorganisms. The microorganisms used for study were S. aureus, S. epidermidis, E. coli, P. aeruginosa, Klebsiella sp, S. typhi, P. vulgaris, Shigella sp, Candida albicans and Aspergillus niger.

Boudjella et al., (2006) also tested pigment against various microorganisms and reported the excellent antimicrobial property. They concluded that pigment could be used as an antimicrobial agent.

2.7.5 Applications of pigment

Krishna et al., (2008) showed in the study, scope for probable application of the bacterial pigment was evaluated for different materials commercially available in the market which included textile, paper, rubber sheets and plastic sheets. The pigment was showed significant results on all materials.

Ali et al., (2011) showed various applications of melanin like lab scale dyeing experiments were carried out on wool fabrics using the produced dyes without mordanting. The lab scale printing experiments were carried out on wool fabrics using the produced dyes without moderating.

Chakraborty et al., (2015) reported in their studies that Bio-pigment can be used as a colorant. They performed an experiment in which they dyed cosmetic product (Lip balm) under lab conditions. The colour formation of the product was stable under room temperature and odour characteristic of the lip balm remained stable throughout the 60 days of testing under all conditions evaluated. The visual aspect was considered uniform under the room temperature.

Kheiralla et al., (2016) mentioned in their research the application of natural pigment isolated from actinomycetes. They performed lab scale dyeing experiment
which was carried out on wool and polyamide 6 fabrics. They showed excellent results and demonstrated successful demonstration of application of pigment at lab scale.