



Isolation, Identification and Optimization of Natural Colorant Producing Soil-borne *Aspergillus niger* and Analysis of the Extracted Pigment

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Authors' contributions

This work was carried out in collaboration among all authors. Author PK designed the study, performed the experiment, wrote the protocol and wrote the first draft of the manuscript. Authors MSW and SLK managed the literature searches and authors AG and SB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Natural colorants have been used in various applications throughout human history, including food, dyes, pharmaceuticals, cosmetics, and various other products. The objective of this study was to separate the naturally occurring filamentous fungus *Aspergillus niger* from the soil and get pigments for prospective industrial uses. Five different soil samples were taken on the campus (botanical garden, polyhouse, agricultural farm) of Padmashri Vikhe Patil College, Pravaranagar, India. By using standard techniques, the *Aspergillus niger* was isolated and identified from the soil samples (morphological & microscopic characteristics). To produce pigment, *Aspergillus niger* was cultured in five different liquid media for 7 days under shaking conditions: potato dextrose broth (PDB), Czapek-Dox broth (CDB), yeast extract malt extract broth (YMB), Sabouraud dextrose broth (SDB), and nutrient broth (NB). The pigments were extracted from the biomass using an ethanol-based extraction technique, and the biomass was then concentrated using a rotary evaporator. Five samples allowed for the isolation of *Aspergillus niger*, and a yield estimate for the brown pigment that was recovered from *Aspergillus niger* was made. Utilizing a UV-VIS spectrophotometer,

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secondary metabolites testing, and spectroscopic analysis of the pigments, it was determined whether they had antibacterial efficacy against test organisms. The composition of the medium had an impact on the pigment. In this study, this species produces more pigment after eight days of culture under various circumstances, including 25°C and pH 4. Carbon and nitrogen are the sources that are necessary for the creation of secondary metabolites and biomass. Temperature, pH, carbon supply, aeration, and fermentation type all affect the pigments because they are by-products of fermentation (solid or submerged). As low-cost, fungi can be used as color production cell factories. The food, pharmaceutical, bio-paint, and textile industries all have emerging uses for fungal pigments.

Keywords: *Natural colorant; biomass; media optimization; brown pigment; secondary metabolites; antimicrobial activity.*

1. INTRODUCTION

The usage of natural colorants that are eco-friendly is the current trend across the globe. In daily life, synthetic and natural dyes are widely employed in many businesses, including those that produce food, textiles, paper, and agriculture practices. The importance of synthetic substances in the environment has increased during the past few decades due to the scarcity and high cost of natural goods in the food business. But the harmful consequences of synthetic items have led to a rise in demand for natural products. Due to their potential for causing cancer, some synthetic colors and chemicals are prohibited products and the impact of their waste disposal on the environment.

“As a result, industries are now available to produce pigments from natural sources and products containing natural compounds that have a high market value. Many active metabolites have been discovered over the centuries from various natural sources such as higher plants, animals, insects, and microorganisms” [1].

“Plants, animals, bacteria, fungi, and algae are all capable of pigment synthesis. Fungi, for example, are known to produce large amounts of pigments. Extracellular enzymes and secondary metabolites produced by fungi include organic acids, pigments, and other food additives” [2].

“Pigments are colored substances that are usually organic or inorganic and are used in specific industries. Pigments are known to cause variations in reflected or transmitted light through wavelength-selective absorption. Pigments are substances that are created by plants, animals, and microorganisms with color are examples of living organisms [3-6]. as a result of selective color absorption indicating normal constituents of cells or tissues adding color even though there

are many natural colors, microbial pigments are more useful in industrial production than related pigments extracted from plants and animals. Because the growth rate of higher plants and animals is generally slower when compared to microorganisms, such productivity for industrially competitive processes is expected. Furthermore, because of its flexibility in the production and down streaming processes, microorganism-produced colorants play an important role as coloring agents. Pigments of various types have been used in food, pharmaceuticals, and other materials” [7].

“Among the fungal groups, ascomycetous and basidiomycetous are known to produce secondary metabolites of various classes of pigments with an extraordinary range of colors. Fungal pigments are secondary metabolites because they are commercially used and made possible by deep-tank fermentation in the same way that antibiotics are mass-produced from fungi. Colors produced by this fungal source under controlled experimentation on a large scale using a wide range of substrates are environmentally friendly” [8]. “Aspergillus, Fusarium, Monascus, Paecilomyces, Penicillium, and Trichoderma are the fungi that produce pigment” [9]. “Most fungi pigments are aromatic polyketide chemical groups such as quinones, flavonoids, melanin, and azophilones. This chemical group of compounds has been discovered to have medicinal applications and could be used as dyes” [10]. Fungal pigments are being more widely used in antibacterial, anticancer, food coloring, and textile dyeing processes. Interestingly, the fungi pigments are natural and do not harm the environment when they are released into it [11].

Since ancient times, natural colorants derived from plants, minerals, and insects have served a variety of uses [12-15]. In many Asian nations,

including Japan and China, these filamentous fungi have been employed for ages to produce rice, wine, soybean cheese, and Anka, which is crimson rice. In East Asia, these colors have long been employed as traditional food additives [16]. The main aim of this study is to isolate, optimize or improve the medium composition for efficient pigment production and analysis of natural colorant-producing filamentous fungi, *Aspergillus niger* from soil and optimized and extract pigment for its potential use, especially for medicinal, food production, bio-paint, etc.,

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples

Soil samples were collected from various places in Padmashri Vikhe Patil College, Pravaranagar, India campus (botanical garden, polyhouse, agricultural farm). The samples were collected from the surface (1-3 cm depth). The collected samples were packed in sterile polyethylene bags and transported to the laboratory. A total of five samples were collected from different areas, collected samples were carried to the laboratory for fungi isolation purposes.

2.2 Isolation of Pigment-Producing Fungi

Soil fungi were isolated by transferring 1gm of soil to test tubes containing 9 mL of distilled water ranging from 10^{-2} to 10^{-5} g/mL. To regulate bacterial growth, 0.1 mL of the diluted soil sample was added to Petri plates containing Potato Dextrose Agar (PDA) and 50 ppm of chloramphenicol to control bacterial growth. For 5-7 days, the plates were incubated at $25\pm 2^\circ\text{C}$ in the fungal incubator.

The single hyphal tip approach was used to isolate the fungal isolates that produce color. Prepared PDA medium was added to sterile Petri plates. Peripheral mycelia fragments were solidified, injected for 5-7 days at 25°C , and checked for fungi colonies that produced color. To store the isolated colonies for future research, they were moved to PDA slants.

2.3 Screening of Pigment-Producing Fungi

Following the isolation of good pigment producers, the pigment potential was tested. The amount of extracellular and intracellular pigment production was used to screen pigment-producing isolates. Among the pigment-

producing fungal isolates obtained from soil, such as *Aspergillus*, *Chaetomium*, *Fusarium*, *Isaria*, and *Penicillium sp.*, *Aspergillus sp.* produced many extracellular pigments. This isolate was chosen for further investigation.

2.4 Identification of *Aspergillus niger sp.*

“The mycelia of the selected fungal isolate were placed on a clean sterile glass slide, a drop of lactophenol cotton blue was added to the mycelia on the glass slide and a new clean cover slip was placed over the glass slide without an airlock. This slide was examined under a microscope and the morphological feature of the fungi was noted at the genus level” [17]. A manual description of fungi was consulted for identification.

2.5 Development of Primary Culture of *Aspergillus niger*

The small amount of inoculum culture was mixed with 5 ml of sterile distilled water and this suspension was inoculated in 50 ml of Potato Dextrose Broth (PDB) and incubated at 25°C for 7 days in a rotary shaker incubator at 150 rpm.

2.6 Optimization of Pigment Production of Fungal Isolate

Because of its high pigment production, *Aspergillus sp.* was chosen for further research. The various parameters that could influence pigment production, such as pH, temperature, carbon and nitrogen source, mineral salts, and inoculum age, were investigated.

2.6.1 Pigment Production on Various Complex Mediums

Different broth media such as potato dextrose broth (PDB), Czapek-Dox broth (CDB), Sabouraud dextrose broth (SDB), nutrient broth (NB), yeast malt extract broth (YEMB) were used for the growth of selected fungal culture *Aspergillus sp.* 50 ml of each broth medium was dispensed in 250 mL Erlenmeyer flasks and sterilized. After cooling, the flasks were inoculated with a fungal mycelial and incubated at $25\pm 2^\circ\text{C}$ for 5 days in a fungal incubator. The growth of fungal colonies in different broth mediums was visually observed and examined. When compared to other broth mediums PDB showed maximum pigment production compared to other liquid media. This medium was used for further studies.

2.6.2 Effect of pH on biomass and pigment production

The initial medium pH for mycelial growth and pigment production was investigated. Potato dextrose broth was prepared and the pH range was adjusted to 4.0, 5.0, 5.5, and 6.5 by adding 0.2 N HCL and 0.2 N NaOH. The medium was added in 250 mL Erlenmeyer flasks and sterilized.

The broth was inoculated with a mycelial and incubated for 7 days at $25\pm 2^{\circ}\text{C}$ in the fungal incubator and triplicates were maintained. The parameters like biomass and extracellular pigments were determined after the incubation period.

2.6.3 Effect of temperature on biomass and pigment production

PDB medium was prepared in an Erlenmeyer flask and sterilized. After sterilization, the mycelial was inoculated and incubated at different temperatures (15, 25, 36, and 50°C) for 7 days under static conditions. Triplicates were maintained. After the incubation period, the parameters such as biomass, and extracellular pigments were studied.

2.6.4 Effect of inoculum age on biomass and pigment production

The age of the inoculum is critical for achieving the best yield. To study the effect of age of inoculum 2, 4, 6, 8, and 10 days were inoculated in a sterilized PDB medium with pH 5.0 and incubated at $25\pm 2^{\circ}\text{C}$ for 7 days in the fungal incubator. After the incubation period, the exact inoculum age of the mycelial and pigment production was determined.

2.6.5 Effect of carbon source on biomass and pigment production

Different carbon sources such as lactose, glucose, sucrose, maltose, and dextrose at 2% (w/v) were supplemented separately in the PDB medium to determine the fungal growth and pigment production studied. The different carbon sources in the medium were inoculated with mycelial and incubated at $25\pm 2^{\circ}\text{C}$ in the fungal incubator for 7 days.

2.6.6 Effect of nitrogen source on biomass and pigment production

Utilizing multiple nitrogen sources, organic and inorganic compounds were used to measure growth and pigment formation. Peptone and yeast extract was employed as organic nitrogen sources, whereas urea, sodium nitrate, and calcium nitrate were used as inorganic nitrogen sources. The PDB medium's pH was set to 5.0 and sterilized before the various nitrogen sources were added. The mycelial was inoculated and cultured for 7 days at $25\pm 2^{\circ}\text{C}$ temperature in a fungal incubator. The generation of pigments and the biomass yield were calculated.

2.6.7 Effect of mineral salts on biomass and pigment production

The effect of different mineral salts such as MgSO_4 , ZnSO_4 , CuSO_4 , KH_2PO_4 , and FeSO_4 at a 2% (w/v) level was added separately to the PDB medium and sterilized. The mycelial was inoculated in the prepared PDB medium containing the different mineral salts and incubated at $25\pm 2^{\circ}\text{C}$ for 7 days in the fungal incubator. After the incubation period, the fungal biomass and pigment production was investigated.

2.7 Pigment Extraction of the Fungal Isolate

2.7.1 Pigment extraction of the fungal isolate by solvent extraction method

"The pigment was extracted from the broth by the solvent extraction method" [18]. "Potato dextrose broth was prepared for the *Aspergillus niger* culture and then incubated for 7 days. After incubating the culture, biomass was separated from broth and the 95% (v/v) ethanol, and on a rotary shaker incubator overnight at 150 rpm and 30°C " [19]. "After this, centrifugation was conducted at 4500 rpm for 15 mins. The same process was repeated for removing the fungal biomass, and the filtration process was done with Whatman filter paper. The extracted colorant was then concentrated using a rotary evaporator at 45°C and 30 rpm to obtain the brown pigment in a semisolid form. After this, centrifugation was conducted at 5000 rpm for 15 mins. The same process was repeated for removing the fungal biomass, and the filtration process was done with Whatman filter paper. The extracted colorant was then concentrated using a rotary evaporator at

45°C and 30 rpm to obtain the brown pigment in a semisolid form” [20,21].

2.8 Analysis of Fungal Pigment

2.8.1 Analysis of fungal pigment by UV-visible spectroscopy

“UV-VIS spectroscopy is used to obtain the absorption spectra of a compound in solute as a solid. UV-VIS spectrophotometer (Model No. ELICO SL 159) was used to identify the pigment’s maximum absorbance (λ_{max}), ranging from 200 to 800 nm” [22,23].

2.8.2 Qualitative chemical analysis

“The crude pigment sample was checked for the presence of secondary metabolites such as alkaloids, phenols, flavonoids, saponins, steroids, cardiac glycoside, and tannins by standard procedure” [24].

2.8.2.1 Cardiac glycoside

Keller-kiliani test was performed to assess the presence of cardiac glycosides. The crude dry powder of the extract was treated with 1 ml of $FeCl_3$ reagent (a mixture of 1 volume of 5% $FeCl_3$ solution and 99 volumes of glacial acetic acid). To this solution, a few drops of concentrated H_2SO_4 was added. The appearance of greenish blue color within a few minutes indicated the presence of cardiac glycosides.

2.8.2.2 Steroids

Liebermann-Burchard reaction was performed to assess the presence of steroids. A chloroform solution of the crude dry powder of the extract was treated with acetic anhydride and a few drops of concentrated H_2SO_4 were added down the sides of the test tube. A blue-green ring indicated the presence of steroids or terpenoids.

2.8.2.3 Alkaloids

The crude extract was evaporated to dryness in a boiling water bath. The residue was dissolved in 2N HCl, the mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with a few drops of Mayer’s reagent; one portion was treated with an equal amount of Dragondroff’s reagent and the other portion was treated with an equal amount of Wagner’s reagent. The creamish precipitate, orange

precipitate, and brown precipitate indicate the presence of respective alkaloids.

2.8.2.4 Flavonoids-lead acetate test

The extract was treated with a few drops of lead acetate solution. The formation of yellow precipitation indicates the presence of flavonoids. Orange to crimson color shows the presence of flavonoids.

2.8.2.5 Phenols

The crude pigment extract was dissolved in 5 ml of distilled water. To these few drops of neutral 5%, ferric chloride solution was added. The dark green color indicates the presence of phenolic compounds.

2.8.2.6 Tannins

The crude extract was treated with an alcoholic $FeCl_3$ reagent. Bluish-black color, which disappears with the addition of a little diluted H_2SO_4 was followed by the formation of the yellowish-brown precipitate.

2.8.2.7 Saponins

The presence of saponins was determined by the frothing test. The crude dry powder of fungal extract was vigorously shaken with distilled water and was allowed to stand for 10 mins. No froth indicates the absence of saponins and stable froth of more than 1.5 cm indicates the presence of saponins.

2.8.3 Antibacterial Activity of the Fungal Pigment of *Aspergillus niger*

“The antibacterial activity was done by the well diffusion method against the test organism. The plates were prepared with the test organism. The wells were cut by using a sterile cork borer, and in each well addition of the extract was done. The diameter of inhibition was determined at 37°C” [25].

3. RESULTS AND DISCUSSION

3.1 Isolation of Pigment Producing Fungi from Soil

The filamentous fungus was successfully isolated from the collected soil, and they were then identified and cultured. The pigment-producing fungi were chosen from the plate’s population

and inoculated on a PDA plate, and the growth pattern was studied for 7 days at 25°C. A variety of distinct fungi that produce pigment were discovered after a subculture of the obtained filamentous fungi was collected. The *Aspergillus niger* colony was initially white and then changed to a variety of shades of yellow, green, brown, or black. It had diameters between 40 and 50 mm and had a velvety or cottony texture.

3.2 Identification of *Aspergillus niger* sp.

3.2.1 Macroscopic Characteristics

The Macroscopic characteristics of pigment-producing fungal strains have been shown in Table 1.

3.2.2 Microscopic characteristics

Using Lactophenol Cotton Blue (LPCB) stain colonies were observed under a 40X microscope, showing the smooth color of conidiospore and conidia as well as hyphae. The conidial heads of *Aspergillus niger* were found to be large and dark brown as observed under a microscope, conidiophores were dark in color

towards the globule. The conidial heads were in a form of biseriate brown but often with separate meters. The vesicles were globose, dark brown, and rough-walled.

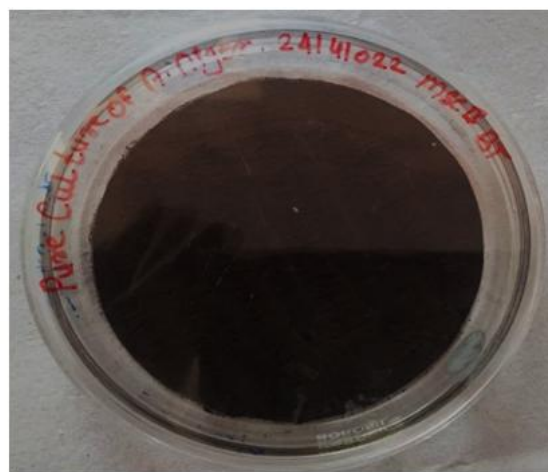
3.3 Optimization of Biomass and Pigment Production of *Aspergillus niger*

3.3.1 Pigment and Biomass Production on Various Mediums

Five different types of liquid media were used potato dextrose broth (PDB), Czapek-Dox broth (CDB), yeast extract malt extract broth (YMB), Sabouraud dextrose broth (SDB), and nutrient broth (NB), were tested for qualification of pigment and biomass. The five mediums showed maximum biomass production in PDB (4.25 ± 0.8 g/L), whereas the SDB showed lower concentrations which is (0.20 ± 0.3 g/L), the pigment production was high in PDB they have components such as metal ions and micronutrients but in other mediums, the only peptone is acted as a source of nitrogen. The total pigment was quantified by determining the absorbance at 510 nm using a UV-VIS spectrophotometer.



(A)



(B)

Fig. 1. Growth of *Aspergillus niger* on PDA medium

Table 1. Macroscopic characteristics

Isolate	Form	Colony color	Reverse side color	Elevation	Lower pattern	Texture
<i>Aspergillus niger</i>	Irregular	Dark brown	Initially white to yellow and then turned black	Unbounded/raised	Smooth	Cottony

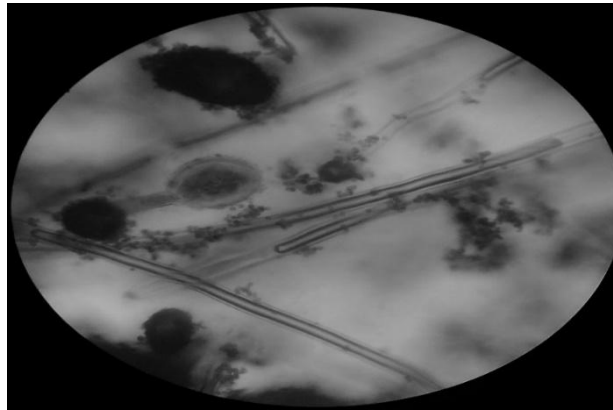


Fig. 2. Microscopically observed conidial head of *Aspergillus niger*

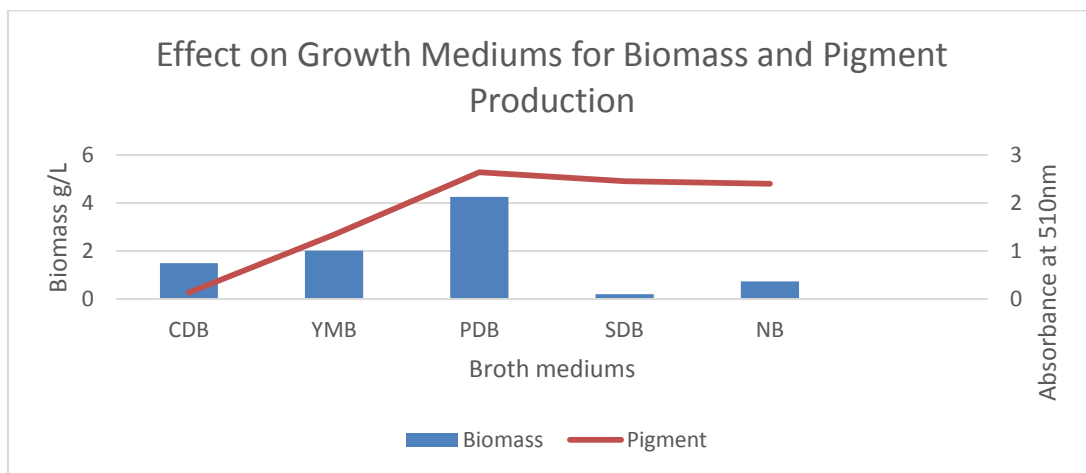


Fig. 3. Effect on growth mediums for pigment production by *Aspergillus niger*

3.3.2 Effect of pH on Biomass and Pigment Production

The effect of various pH (4, 5, 5.5, 6.5) on biomass and pigment production was studied. A maximum pigment production of 1.58 nm and a high biomass concentration of 4.75 g/L were observed at pH 4 after 7 days of incubation. In the case of pH 5, 5.5, and 6.5 pigment production was 1.32 nm, 1.28 nm, and 1.43 nm respectively. Generally, fungi prefer acidic pH for good growth and activity. It can be observed from this experiment that at pH 4.0, the production of biomass and pigment production was high (Fig. 4.)

3.3.3 Effect of temperature (°C) on biomass and pigment production

The influence of various temperatures (15, 25, 36, and 50°C) on biomass and pigment production of the fungal isolate was studied. The

incubation temperature of 25°C showed maximum pigment production of 2.68 nm and biomass of 5.05 g/L (Fig. 5). Higher temperatures reduced pigment production and biomass yield. From the results, it was observed that the temperature of 25°C appeared to be optimum for the growth of the fungi and secretion of pigments.

3.3.4 Effect of inoculum age on biomass and pigment production

The culture was grown in a PDB medium by different inoculum ages from 2-10 days. A maximum pigment production of 1.34 nm and biomass production of 5.5 g/L (Fig. 6) was achieved at the age of inoculum day 10. There was a slight decrease in biomass and pigment production and the least amount of pigment production was observed on day 2 age of the inoculum.

3.3.5 Effect of carbon source on biomass and pigment production

The effect of carbon sources (glucose, sucrose, maltose, lactose, and dextrose) in the culture medium for biomass and pigment production in 2% (w/v) concentration was studied. At 510 nm absorbance, maximum pigment production by *Aspergillus niger* with dextrose, as a carbon source was found to be 1.42 nm in Potato Dextrose Broth (PDB) medium, and glucose gave the second highest pigment production of 0.49 nm. The mycelial biomass yield by *Aspergillus niger* recorded a maximum production of 5 g/L (Fig. 7) in the fermentation medium by using dextrose as a carbon source. The minimum was recorded in maltose (0.17 nm). In general, dextrose was found to be a good source of all the sugars used except maltose which showed less pigment production.

3.3.6 Effect of nitrogen source on biomass and pigment production

In this study, the isolated fungus was grown in Potato Dextrose Broth (PDB) with different organic nitrogen sources such as peptone, yeast extract, and inorganic sources of nitrogen such as urea, sodium nitrate, and calcium nitrate for biomass and pigment production in 2% (w/v) concentration. Among the organic nitrogen sources, yeast extract gave the highest yield of 3.6 g/L mycelial growth and 0.98 nm pigment production. Using inorganic nitrogen sources, calcium nitrate gave a total mycelium growth of 4.9 g/L and pigment production of 1.34 nm. Minimum biomass production of 0.7 g/L was obtained while using urea and minimum pigment production was observed as 0.35 nm (Fig. 8). In general, all the nitrogen sources can support pigment production. Among these, inorganic source of nitrogen was found to be better than organic nitrogen source for pigment production.

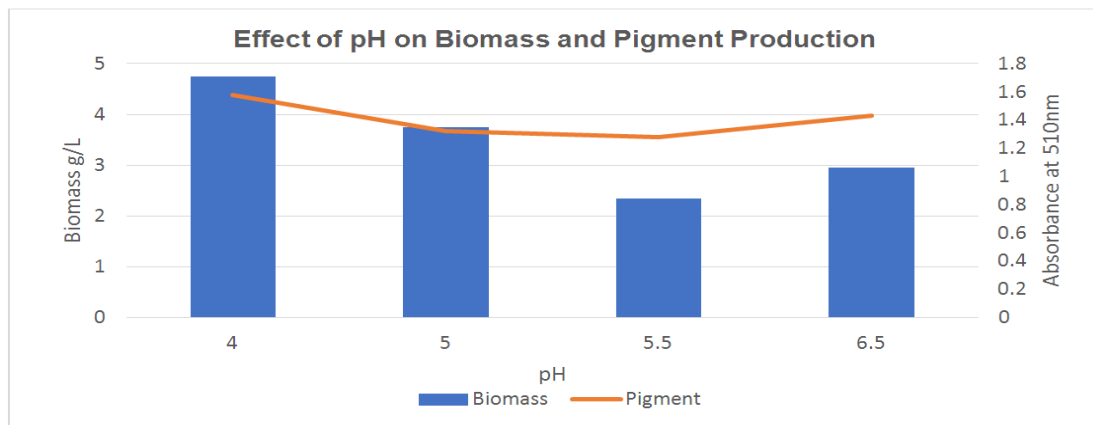


Fig. 4. Effect of pH on biomass and pigment production of *Aspergillus niger*

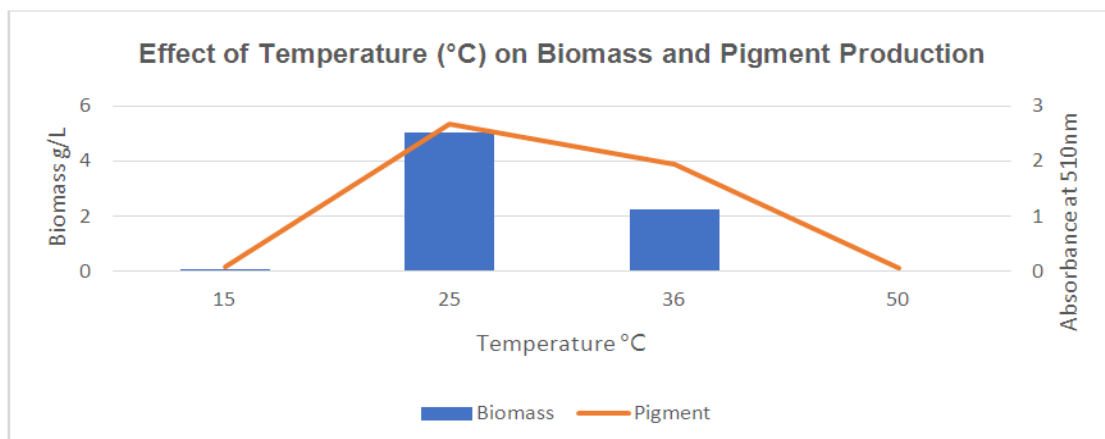


Fig. 5. Effect of temperature (°C) on biomass and pigment production of *Aspergillus niger*

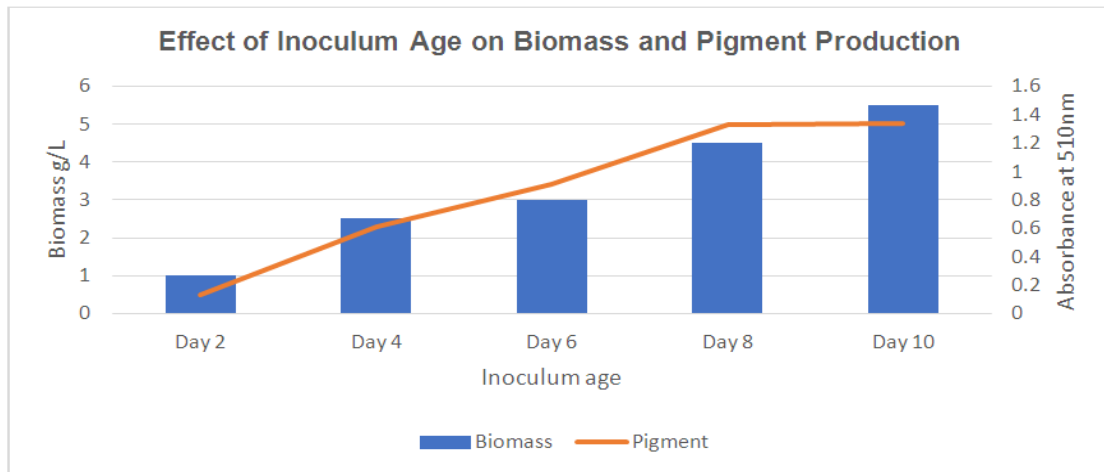


Fig. 6. Effect of inoculum age on biomass and pigment production of *Aspergillus niger*

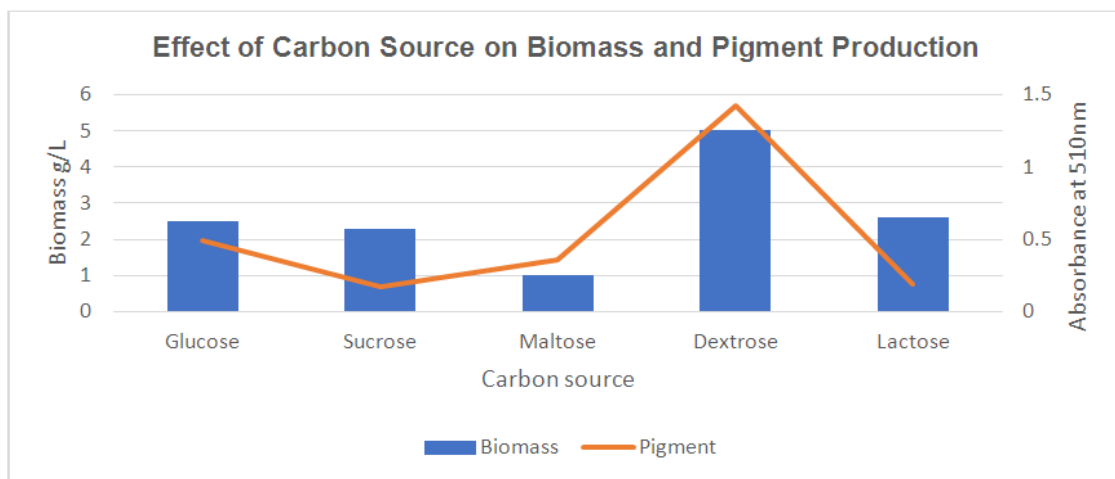


Fig. 7. Effect of carbon source on biomass & pigment production of *Aspergillus niger*

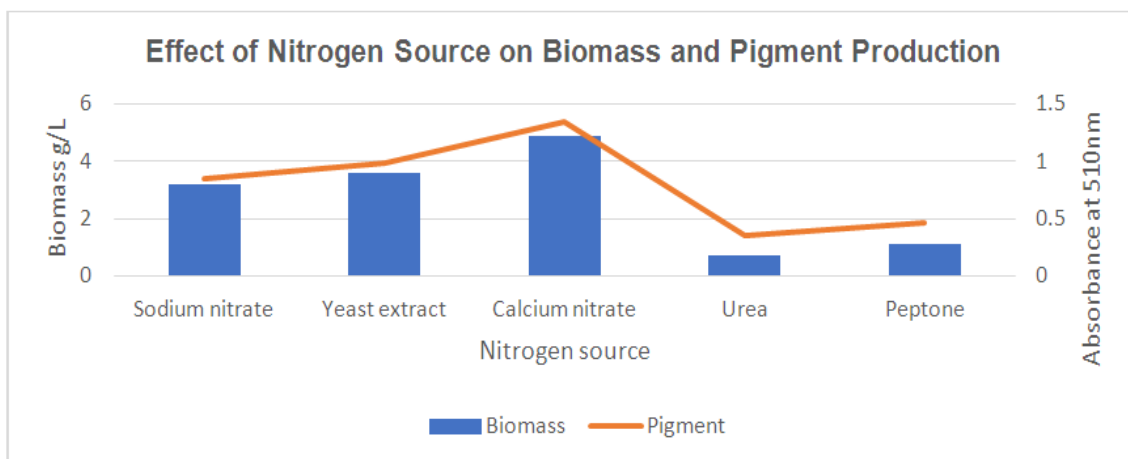


Fig. 8. Effect of nitrogen source on biomass and pigment production of *Aspergillus niger*

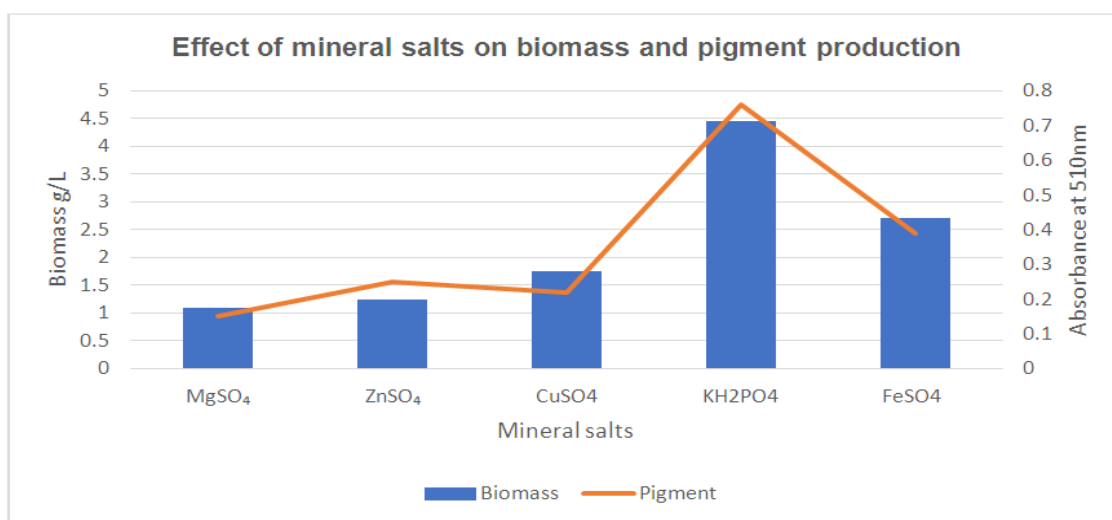


Fig. 9. Effect of mineral salts on biomass and pigment production of *Aspergillus niger*

3.3.7 Effect of mineral salts on biomass and pigment production

The influence of mineral salts in 2% (w/v) concentration such as MgSO₄, ZnSO₄, CuSO₄, KH₂PO₄, and FeSO₄ on biomass and pigment production was studied. Among these mineral salts, the fungal isolate showed better pigment production in KH₂PO₄ (0.76 nm) and biomass yield of 4.45 g/L in the PDB medium (Fig. 9). Minimum pigment production of 0.15 nm and mycelial growth of 1.1 g/L was observed in MgSO₄.

3.4 Analysis of Fungal Pigment

3.4.1 Spectrometric analysis

The UV Visible spectrum of purified pigment showed a broader absorption spectrum from UV

to visible region. The 0.1 ml of crude extract was dissolved in (2 ml, 4 ml, 5 ml & 10 ml) of ethanol, and ethanol was used as a negative control. This absorption spectrum showed in the graph (Fig. 10). The value of absorption decreased towards the visible region. The absorbed characteristic of the brown pigment was like the UV-VIS absorption spectra of the melanin pigment.

3.4.2 Qualitative chemical analysis

Preliminary qualitative chemical analysis of the crude ethanolic extract of *Aspergillus niger* pigment sample was checked for the presence of secondary metabolites by standard protocol and the results were tabulated in Table 2.

Table 2. Qualitative Chemical Analysis [Positive (+) Negative (-)]

Sr. No.	Chemicals	Results
1	Glycosides	+ (brown to greenish blue color)
2	Steroids	+ (blue-green ring indicates terpenoids)
3	Alkaloids	
	• Mayer's reagent	+ (creamish ppt form)
	• Wagner's reagent	- (no orange ppt form)
4	Flavonoids	+ (orange to crimson color)
	Lead acetate test	
5	Phenols	- (no dark green color form)
	Ferric Chloride test	
6	Tannins	+ (yellowish brown ppt form)
7	Saponins	No Foam Formation

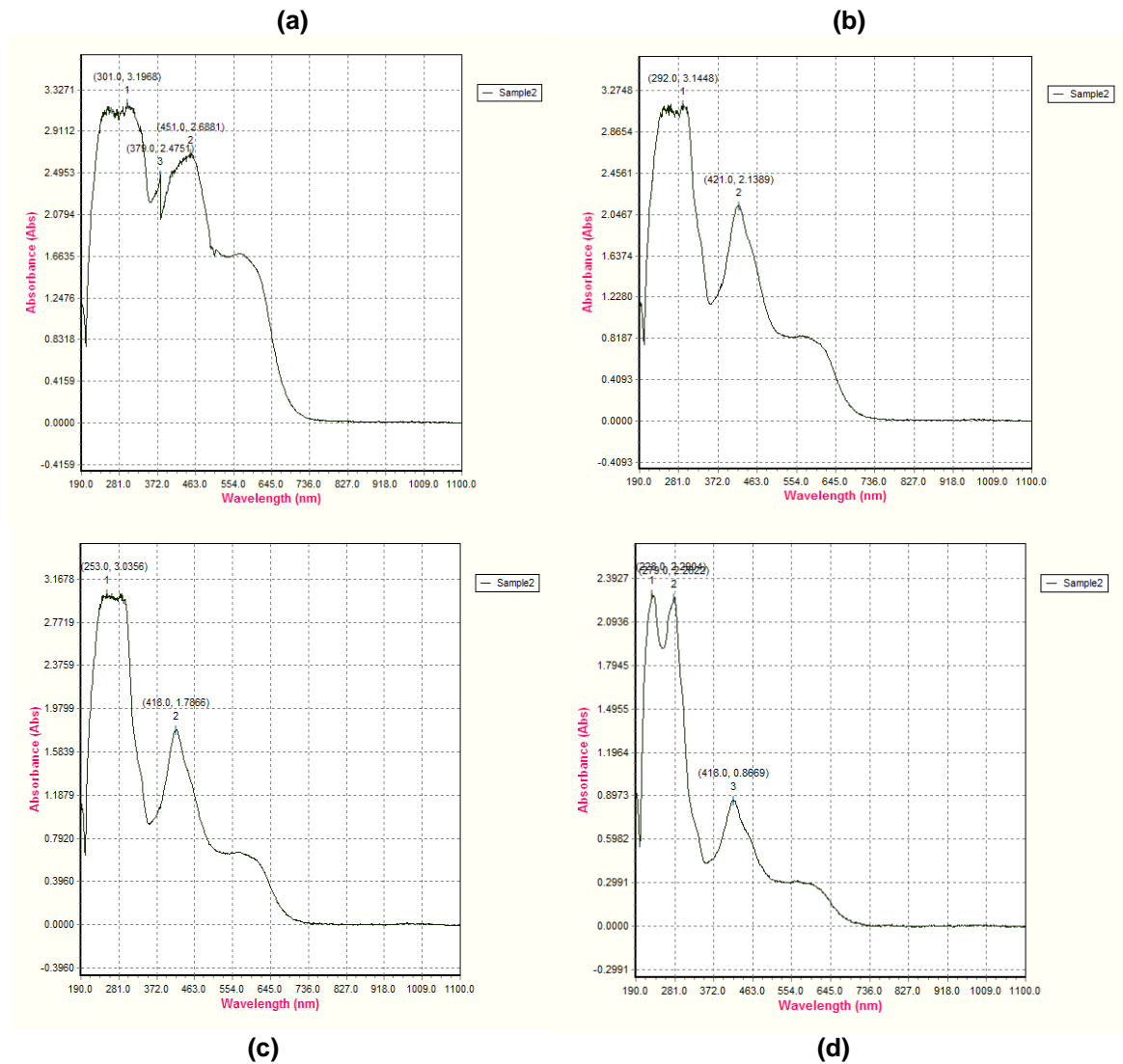


Fig. 10. Absorbance value at different concentrations [a) 0.1 ml crude extract diluted with 2 ml ethanol, b) 0.1 ml crude extract diluted with 4 ml ethanol, c) 0.1 ml crude extract diluted with 5 ml ethanol, d) 0.1 ml crude extract diluted with 10 ml ethanol]

Table 3. Antibacterial Activity: Zone of Inhibition

Sr. No.	Pathogens	Ethanolic extract zone of inhibition in (mm)
1	<i>E. coli.</i>	15
2	<i>Staphylococcus aureus</i>	17

3.4.3 Antibacterial activity of the fungal pigment of *Aspergillus niger*

The antibacterial activity of the pigment produced by *Aspergillus niger* was tested against the selected bacterial species (*Escherichia coli* and *Staphylococcus aureus*) and the results were summarized in Table 3. The results indicate that *Aspergillus niger* pigment exhibited good activity against *Staphylococcus aureus*

(17 mm) and medium activity against *E. coli* (15 mm).

4. CONCLUSION

This study's main objective was to isolate pigment-producing fungus from soil and extract fungal pigment as a source of antibacterial activity and various industrial applications. Owing to the contradictory reports on the safety of

synthetic dyes and due to the indiscriminate use of non-permitted colors, there is an important need to identify natural pigment sources as safe colorants. It is known that fungi isolated from soil-produced pigments are used for various industrial applications. The use of fungal pigments is gaining accessibility in textile dyeing, food colorant, antimicrobial and anticancer activities. Soil samples were collected and pigment-producing fungi were isolated and characterized. An antibacterial activity test was done and assessed. The results of different experiments are summarized below.

A total of 5 soil samples were collected from different areas of the Padmashri Vikhe Patil College, Pravaranagar Campus (botanical garden, polyhouse, agricultural farm) and analyzed for their fungal growth and pigment production. As the isolates of fungi were found to have pigment production and there is a need for a vigorous screening of these isolates for higher pigment production. The potent pigment-producing isolates were identified morphologically at the genus level as *Aspergillus niger* sp. and were used for further studies because they have a high diffusing ability in the medium.

The optimal conditions for maximum pigment production, the selected fungal isolate *Aspergillus niger* was grown at different pH, temperature, carbon and nitrogen sources, mineral salts, inoculum age, and different broth mediums. The biomass and maximum pigmentation were seen in Potato Dextrose Broth (PDB) medium. The optimal pH of 4, the temperature of 25°C, dextrose as carbon source, calcium nitrate as nitrogen source, KH_2PO_4 as mineral salt, and 10 days of inoculum age supported good biomass and pigment production.

By solvent extraction, the pigments from the fermented broth were extracted using ethanol. The maximum absorbance (λ_{max}) of the pigment was identified by a UV-VIS spectrophotometer and the UV spectrum exhibited absorption bands.

The compound that was produced by *Aspergillus niger* revealed that the pigment has antibacterial activity. The pigment was tested against the selected bacterial species *Staphylococcus aureus*, and *Escherichia coli*. The results indicated that *Aspergillus niger* pigment exhibited good activity against *Staphylococcus aureus*

(17 mm), and medium activity against *Escherichia coli* (15 mm).

From the preceding summary, *Aspergillus niger* provides excellent opportunities for the industrial production of bio-colorants and its role in antibacterial activity and various potential industrial applications.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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