Original Article

Optimization And Production Of Bio-Colorant From Fungi

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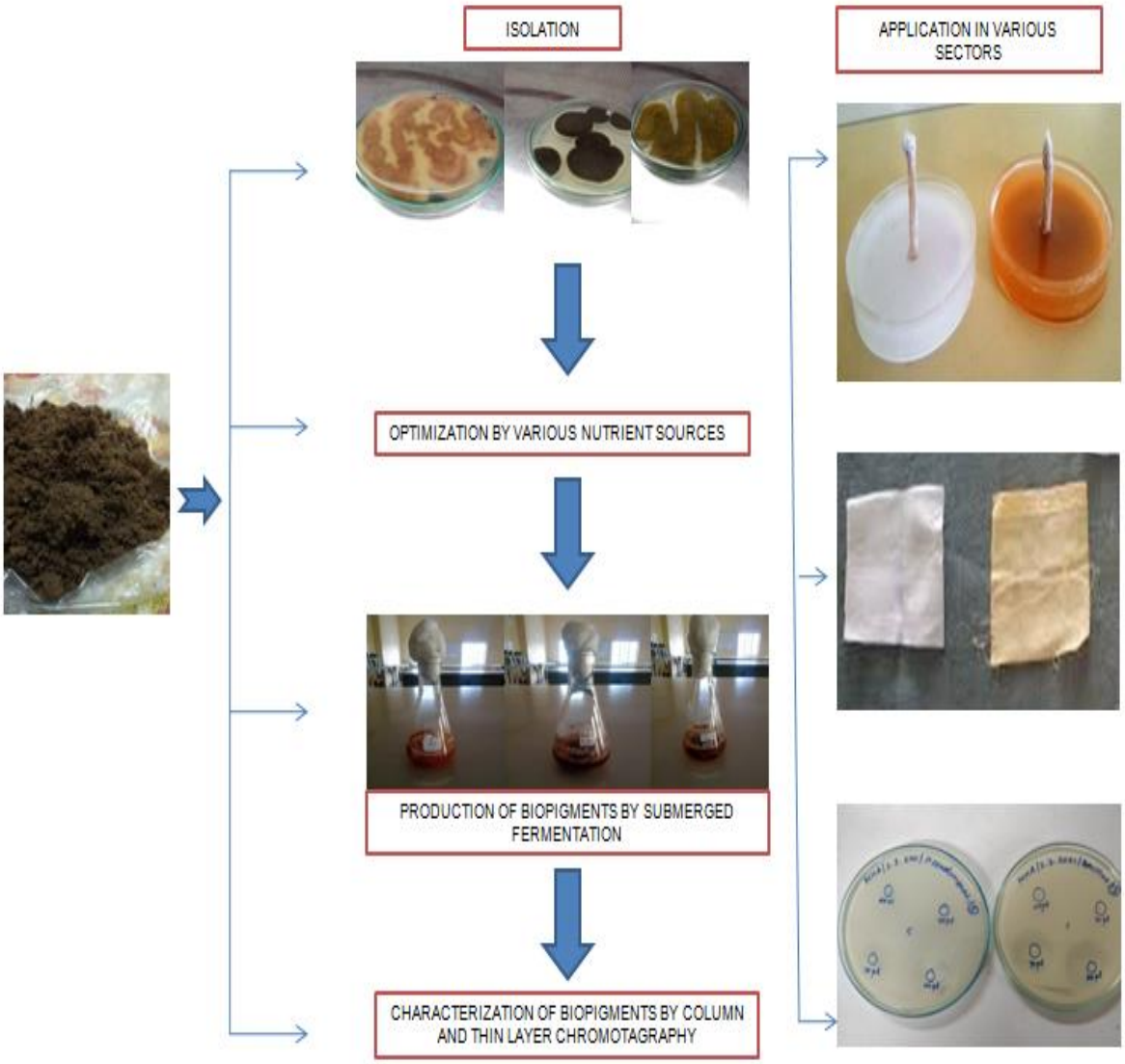
GRAPHICAL ABSTRACT:



Abstract

Background: Fungi are biologically active producers of pigmented compounds that can be used as food product additives or as an antibacterial constituent in food and pharmaceutical industries. It is a powerhouse of bioactive secondary metabolites, and many of these compounds have been found to have important applications in the antibiotics and food preservation industries.

Objective: Aim of the research is to exploit the indigenous diversity of the fungal population for biocolornant production and their application in the pharmaceutical, wax, and textile industry.

Materials and method: From the sample source primary isolation of pigment-producing fungi were isolated. Further optimization of the medium for pigment production was carried out based on different carbon, nitrogen, mineral salts, pH, and temperature. Extracted crude pigment was characterized and purified by the solubility with different solvents, TLC, and column chromatography. The potential activity of the pigment is confirmed by the antibiotic sensitivity test and coloring of the candle and cloth.

Results: In our study, we isolated the fungi from the garbage soil. Out of 10 isolates from which 3 were selected for the study. Then the selected isolates are identified by microscopic examination and confirmed as Aspergillus sp., and Monascus sp. Optimization studies were carried out to enhance pigment production. The optimized condition for the production of pigment was best in the presence of glucose, peptone, magnesium sulfate as carbon, nitrogen, and mineral salts sources. The maximum growth is attained at the pH of 4 and temperature 25-degree Celsius. The extracted pigment was purified using column chromatography by collecting the 15 fractions in each isolate. Among the various fractions, fraction 3 (orange J1), fraction 5 (J2), and fraction 5 (J3) show only one spot in the Thin Layer Chromatography retention factor value is 0.4 and 0.6. The antibacterial activity of the crude extract was subjected to the agar well diffusion method at different concentrations (25µl,50µl,75µl,100µl). Each crude extract was tested against the gram-positive organism of (Bacillus cereus) and the gram-negative organism (Pseudomonas aeruginosa) bacteria. The results of J2 and J3 were observed as the zone of inhibition against the targeted strains at 75µl and 100µl.

Conclusion: This preliminary highlight of the study is to reveal that fungal pigments J2 and J3 has the ability of antibacterial activity and coloring of J1 isolate in candle and cloth which is effective in dying textile.

KEYWORDS: Bioactive pigments, Aspergillus sp., Monascus sp., Thin layer chromatography, textile dying, zone of inhibition

1. INTRODUCTION

Chroma plays a vital role in our life through clothes, furniture, and the attractiveness of food[16]. [16]The increasing demand for natural chromatic colors leads to the production of coal tar dyes. The owing demand for colors leads to the production of numerous artificial and synthetic colorants which can be used as coloring agents in food products, dying textiles, cosmetics, and the pharmaceutical industry. These synthetic dyes give an adverse life-threatening effect on the soil environment. Biochromes are substances produced from microorganisms that have extensive applications in the pharmaceutical, food, and textile industries with increasing demand consumers for biochromes. Biochromes have a broader clinical benefit for antioxidant, anticancer, antibiotic, and immunosuppressive, treatment for diabetes mellitus and other diseases. The chief origin of biochromes is from the flora, fauna, and microorganisms. The use of floral pigments has drawbacks because of inaccessibility throughout the year and pigment stability and solubility.

Fungi are the best alternative source which is good and readily available for the biological pigments which have the advantage of being attainable and quick growth in a low-cost culture medium, for the synthesis of pigments with various tinge shade, secure, dissolvable pigments and conventional processing for large scale production in industries[2]. Fungi release a large number of organic compounds as secondary metabolites [21], which is helpful in cellular processes by transcription and development of intercellular communication. The improvement or enhancement of fungal pigment production yield can be achieved by advanced techniques like [1] Genetic manipulation in the pigment production pathway [2] molecular screening technology and bioinformatics study help to improve and ameliorate the gene expression and accretion of unusual metabolites [3] Optimization strategy for the improvement of fermentation conditions for pigment production. Due to the demerits of chemical colorant, it increases public awareness, and eco-friendly, and health concerns also strict environmental and ecological rules and regulations, have challenged researchers to undertake both qualitative and quantitative research on pigments derived from fungi, having a minimal ecological disadvantage. The need to exploit novel and safe biochromes from vast diverse taxonomy groups of fungi populations for the present demand of eco-friendly pigments using appropriate techniques. The main aim of this study is to exploit the indigenous diversity fungal population for biocolornant production and their application in the pharmaceutical, wax, and textile industry.

2 MATERIALS AND METHODS

2.1 Procurement of soil sample

The soil samples were from a garbage dump site in the local area near Pollachi, Coimbatore district, Tamil Nadu, India. Soil samples were collected by scraping the soil surface (up to 10-20 cm depth). Collected samples were transferred in sterile polythene zip-lock bags and brought carefully to the laboratory and then stored under aseptic condition.

2.2 Isolation of fungi strains:

For isolation of the fungi, the potato dextrose agar was prepared, sterilized, and plated. About one gram of the collected garbage soil was taken and serially diluted up to 10^{-7}. Dilution of 10^{-2}, 10^{-3}, $10^{-4,}$ and 10^{-5} was plated by spread plate technique. Then the plates were incubated at 25ºC for 3 to 4 days after the colonies were observed[1].

2.3 Optimization of fungal strains:

The optimization of produced fungal pigment from the isolates was carried out in Potato Dextrose Broth. This process was carried out to increase the pigment production and growth rate. Temperature and pH are important parameters for the growth of pigment-producing fungi[11].

2.3.1 Effect of carbon sources

The ascendancy of different carbon sources supplied with (2%) of glucose, fructose, lactose, maltose, and sucrose in the separated medium and pigment production was observed. The freshly subcultured fungal strains of J1, J2, and J3 were inoculated to the potato dextrose broth supplemented with different carbon sources and incubated at the optimum temperature of 25°C for 5 days without agitation.

2.3.2 Effect of nitrogen sources

Influence of different nitrogen sources of each (1%) peptone, yeast extract, ammonium nitrate, sodium nitrate, and sodium nitrite separated medium for determination of pigment production. The freshly subcultured fungal strains of J1, J2, and J3 were inoculated to the PD broth implemented with different nitrogen sources, and the plates were incubated at the optimum temperature of 25°C for 5 days without agitation in the shaker.

2.3.3 Effect of mineral salts

The Potato Dextrose broth was supplemented with 0.5% of different salts including magnesium sulfate ($MgSO_4$), zinc sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), dihydrogen potassium phosphate (KH_2PO_4) and ferric chloride ($FeCl_2$). Fungal strains were inoculated in the mineral salt supplied with potato dextrose medium and incubated for 5 days at the optimum temperature. Pigment production was checked on the 5th day of the incubation period and the results obtained were recorded as previously described.

2.3.4 Effect of pH

The fungal isolate was inoculated in sterile Potato Dextrose Broth broth with different pH 2, 4, 6, 7, and 8.0 and was incubated at 27°C for 5 days. An assay for pigment production was carried out. The optimum pH achieved by this step was fixed for further experiments. The mycelial biomass yield was estimated by washing with de-ionized water and drying at 50ºC for 48 h[14].

2.3.5 Effect of temperature

The isolated fungi were inoculated in the sterile Potato Dextrose Broth and incubated at different temperatures of 20, 25, 30, 35, and 40ºC for 5 days. The optimum temperature was achieved by this step and fixed for further experiments. The fungal mycelia biomass yield was determined by washing it in the de-ionized water and kept it for drying at 50ºC for 48 h[14].

2.4 Production of fungal pigments [12]:

The production of J1, J2, and J3 were carried out on the basis of optimization done earlier. The composition of media for the production of J1, J2, and J3 is 2% glucose, 1% peptone, and 0.5% magnesium sulfate at pH 4, and temperature 25degree Celsius.

2.5 Extraction of fungal pigments

2.5.1 Centrifugation

The extracellular pigment produced from the fungal strains was filtration (Whatman filter paper No. 2) and followed by liquid-liquid extraction (Lu et al., 2018). The 5 days the fungal mycelium was removed and the medium was subjected to a centrifuge of 4000rpm for 15

min. Then the supernatant was taken and checks the solubility with solvent (hexane, ethyl acetate, petroleum ether, chloroform, butanol, and acetone). The solubility of the obtained compound was in acetone.

2.5.2 Direct crystallization method

The supernatant collected from the fungal media is purified by a direct crystallization process. The supernatant was measured and an equal volume of distilled water was added to it and mixed well. The mixed solutions were to crystallize by air drying.

2.6 Characterization of fungal pigments

2.6.1 Column chromatography**:**

The crude compounds were dissolved in the acetone in equal volumes and subjected to separation through column chromatography. For the stationary phase, the silica slurry was prepared with the same solvent in the ratio of 1:2. The fractions were eluted with acetic acid- ethyl ether (1:10)[5].

2.6.2 Thin layer chromatography**:**

The biologically active pigments present in crude extract after filtration was subjected analyzed to determine the compounds by Thin Layer Chromatography using silica gel embedded plates. The crude extract obtained after column chromatography was suspended in an equal volume of nonpolar solvent and placed a spotted at the bottom of the TLC sheet using a glass capillary tube. The TLC was run using a mobile phase solvent of Butanol: glacial acetic acid: water in a 40:10:10 ratio. The separated spots in which compounds traveled were observed under the naked eye using iodine vapors[9]. R_f values of the separated bioactive compounds in crude extract were measured by using the adopted standard formula.

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent system}}$$

2.7 Screening the antibacterial activity of fungal pigments:

2.7.1 Determination of antibacterial activity by Minimum Inhibitory Concentration (MIC) method

The antimicrobial activity of fungal pigment was assessed by determining the MIC values. Then Broth dilution method was used to determine MIC against Pseudomonas aeruginosa and Bacillus cereus. The pigment was taken at concentrations of 5, 10, 20, 40, and 80mg/mL was then mixed with sterile Muller Hinton Broth. The tubes were incubated at a temperature of 37°C for a time period of 24 hours to evaluate the MIC of fungal pigments[8].

2.7.2 Screening of antibacterial activity of fungal pigments by Kirby Bauer method

The antibacterial activity of isolates fungal pigments was tested by the Kirby Bauer method. In this antibiotic susceptibility method, Muller Hinton Agar (MHA) was prepared and the well was placed in plates uniformly lawn culture was performed using the targeted pathogen. The crude extract was loaded in the wells with different concentrations, and plates are incubated at 37ºC for 24 hrs without inverting the plates. Growth was observed after 24hrs[8].

2.8 Effectiveness of pigment in candle

Caustic soda (purified NaOH- 1 cup or 10g) has been taken in a glass beaker and mixed with doubled amount of distilled water (2 cups). Mix it well and allow it to cool. Then add wax in the same glass beaker (7 cups) mix it well and add pigment (Orange color) as powder form mix it well, using a magnetic stirrer for up to 30 minutes. If the pigment is not evenly spread keep mixing. Finally, pour the mixture into the mold and keep it in the refrigerator at 4°C for overnight. The next day we get a wax with orange pigment.

2.9 Effectiveness of pigment in cloth

The cotton fabric sample was scoured in distilled water at 60ºC. Mordant: Soak cotton fabric sample was pre-mordant using 2% ferric chloride in a conical flask at 60ºC for 30 min and allowed to cool overnight. Dyeing: 1g of fabric was dried in 50ml of filtered pigment [9].

3 RESULTS AND DISCUSSION

3.1 Procurement of soil sample

The environmental soil has been stressed due to pollution but it contains a majority of microorganisms that were prevalent in producing different metabolites for their survival between microbial communities. The sample was collected from a garbage site which is dry soil and brown in color (Table. **1**)

S.NO	SAMPLE	SAMPLE SITE	SAMPLE COLOUR	NO. OF ISOLATES	ISOLATES TAKEN FOR SCREENING
1	SOIL	GARBAGE DUMP SITE	DRY, BROWN	10	3

Table 1 represents the pre-analysis of the sample before processing for the identification of pigment

3.2 Isolation of the fungal strains

From the garbage soil, the various fungal colonies were isolated and screened its pigment production. According to [17]colonies that exhibit discrete pigment should be selected and cultured for pigment production. So based on high pigment color intensity, three biologically active pigmented colonies were selected. Among the various pigmented fungi, extracellular pigment-producing fungal strains labeled as J1, J2, J3 (Figure **2**) were selected for further studies and Table 1 represents the detailed note of the plate colony morphology seen in the naked eye.

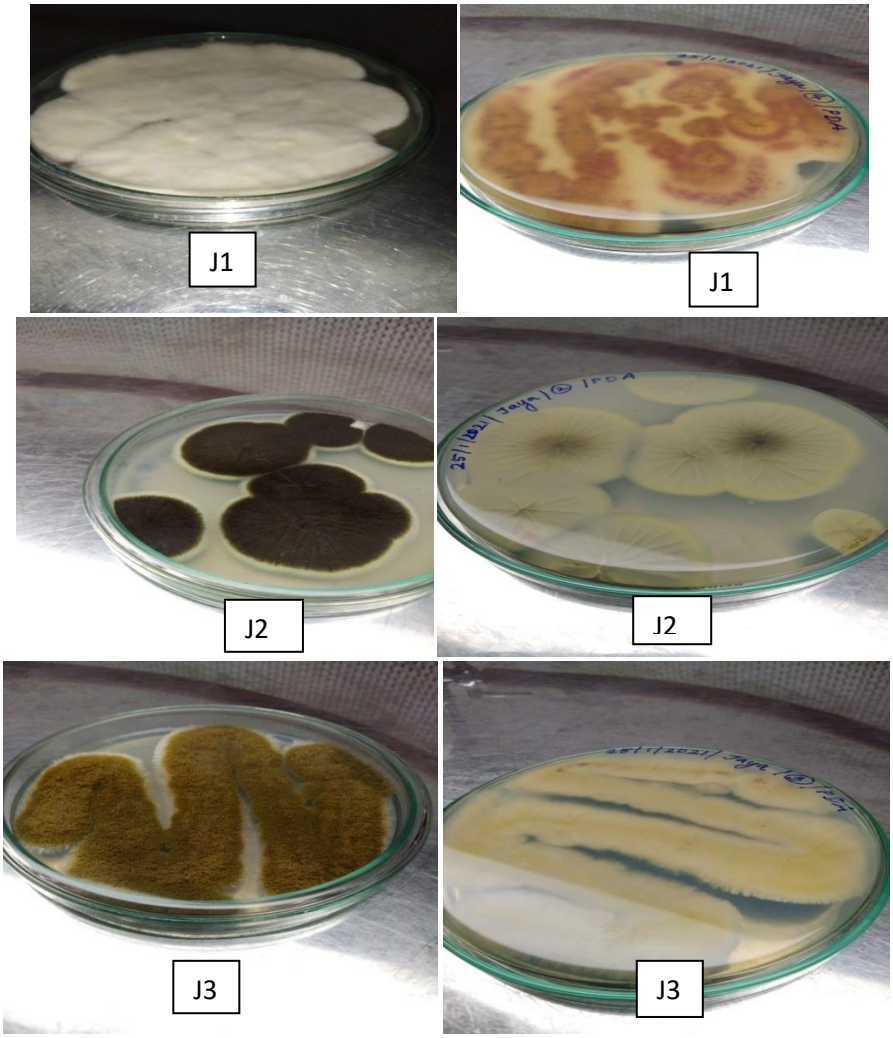


Figure 2 Culture plate of J1, J2, and J3

3.2.1 Macroscopic morphology

The macroscopic characteristics of all three isolates J1, J2, and J3 were observed (table **2**).

ISOLATES	**J1**	**J2**	**J3**
FORM	IRREGULAR	CIRCULAR	CIRCULAR

COLONY COLOR	WHITE	BLACK	BROWN
REVERSE SIDE	REDDISH ORANGE	HEAVILY FURROWED WITH VENATION	RADIALLY FURROWED
ELEVATION	RAISED	FLAT	FLAT
TEXTURE	COTTONY	POWDERY	POWDERY
SPORULATION	NO	HEAVY	HEAVY
FUNGI	M0NASCUS SP.,	ASPERGILLUS SP.,	ASPERGILLUS SP.,

Table 2. Colony morphology of J1, J2, and J3

3.2.2 Microscopic morphology**:**

Microscopic characteristics of fungal strains were studied under a light microscope at 40X and 100X magnification. According to the morphological characteristics [15]we observed different characteristics and observed its microscopic view. After the Lactophenol cotton blue mount of (Figure **3**) fungal strain, it is observed with the 10X objective of J2 and J3 appear as hyphae with conidiophores and conidia resembling the structure of the plant. And J1 appears as hyphae.

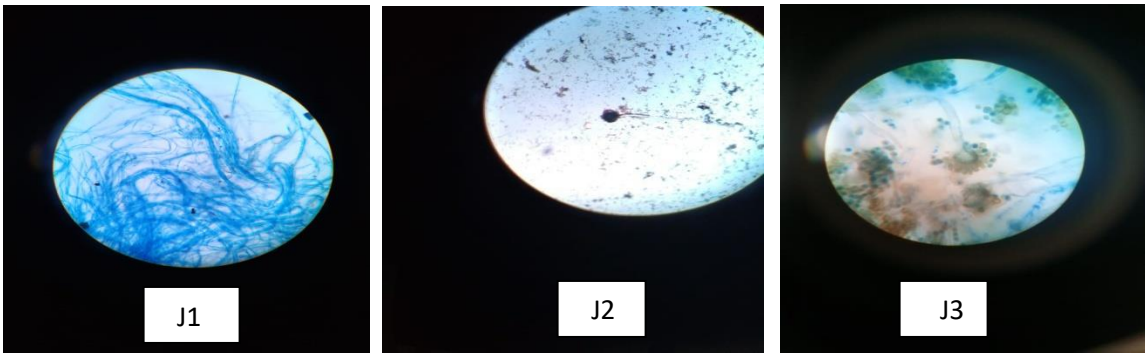


Figure 3. Microscopic observation of J1, J2 and J3

3.3 Optimization of fungal strains

3.3.1 Effect of carbon sources

Additional glucose as a Carbon source in the potato dextrose broth increases the biomass production in all the isolates when compared to the control medium having only potato dextrose broth without additional carbon sources. This was followed by sucrose (J2 and J3) and lactose (J1). In contrast, maltose, as an additional Carbon source restricted the biomass production for J1. Other carbon sources were moderately supportive of the growth of the isolates in PD broth. It is concluded that Glucose was the effective carbon source for the high yield of the isolates. [7]has stated that maltose was more effective than fructose and lactose (Figure **4**).

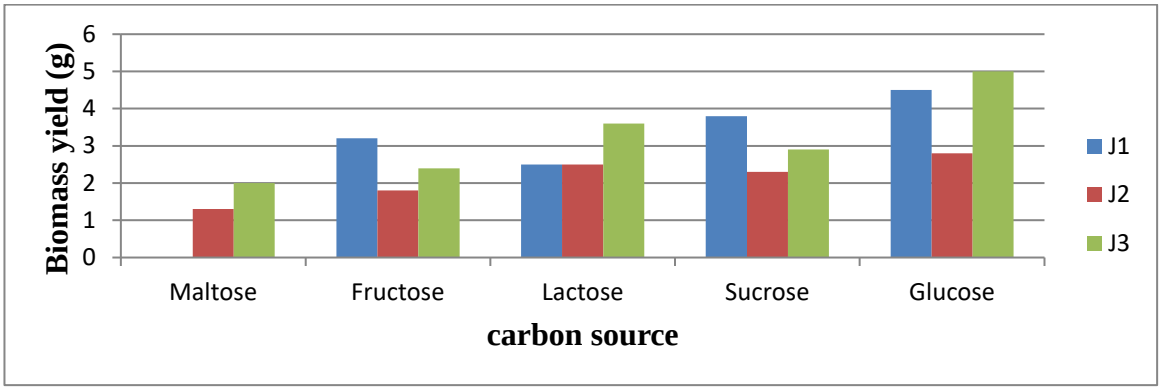


Figure 4 Effect of different carbon sources J1, J2, and J3 strains where the x-axis represents the carbon source of fungal medium and the y-axis represent the cell dry weight (grams) after the 5 days of the incubation.

3.3.2 Effect of nitrogen sources

Five different nitrogen sources were tested for optimization of nitrogen sources. Out of various nitrogen sources tested, peptone enhanced higher biomass production from isolates J2 and J3. Similar to the peptone results maximum biomass was also observed in a medium supplemented with yeast extract for the J1 isolate. Media with sodium nitrite, sodium nitrate, and ammonium nitrate supported moderate biomass production for all the isolates (Figure **5**). [7]has stated that peptone supported maximum biomass production and inhibited the growth of fungi and pigment production were visualized after supplementing the medium with sodium nitrite.

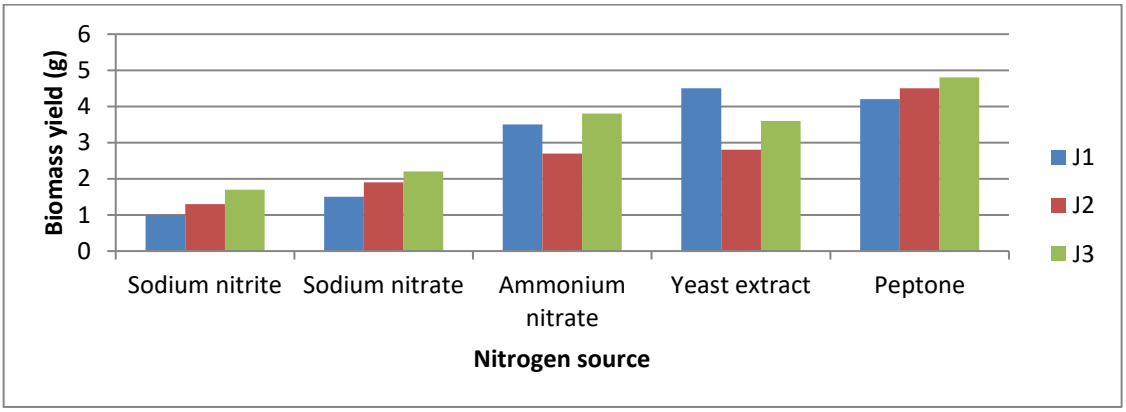


Figure 5 Effect of different nitrogen sources of J1, J2, and J3 strains where the x-axis represents the nitrogen source of fungal medium and the y-axis represents the cell dry weight (grams) after the 5 days of the incubation.

3.3.3 Effect of mineral salts

For the different mineral salts tested, only dihydrogen potassium phosphate and magnesium sulfate increased biomass production from J1, J2, and J3. Maximum biomass production in presence of KH_2PO_4 was observed in J1 and $MgSO_4$ was observed in J2 and J3. Other mineral salts like Zn^{2+}, Cu^{2+}, and Fe^{2+} show inhibited biomass production (Figure **6**). [7]has stated that biomass produced in the presence of magnesium sulfate was found to be highest in 10 days of incubation followed by dihydrogen potassium phosphate, whereas other mineral salts such as Zn^{2+}, $Cu^{2+,}$ and Fe^{2+} showed no visible growth of biomass production.

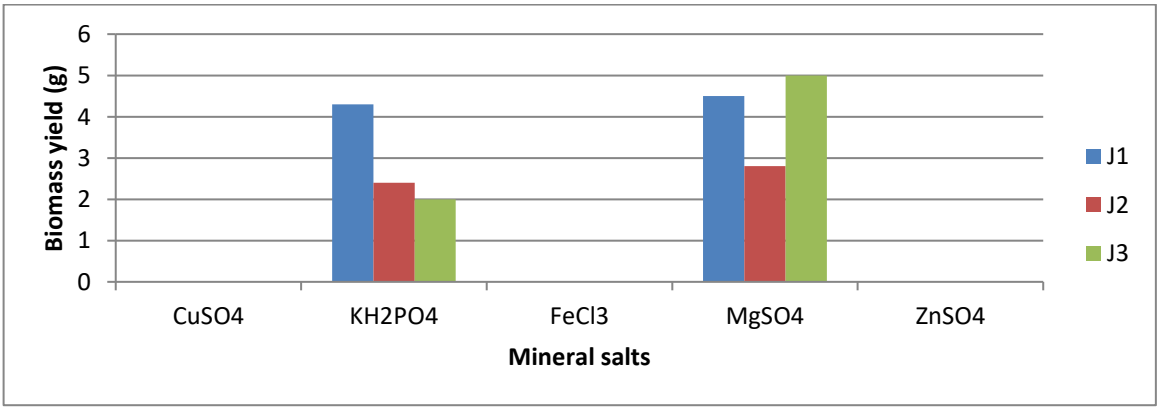


Figure 6 Effect of different mineral salts of J1, J2, and J3, where the x-axis represents the mineral salts of fungal medium and the y-axis represents the cell dry weight (grams) after the 5 days of the incubation.

3.3.4 Effect of pH

In order to evaluate the effect of pH on mycelia growth and pigment production, isolated fungi were cultivated at different initial pH values (2, 4, 6, 7, 8) in shake flask cultures. The optimal initial pH for both mycelia growth and pigment production is found that optimum pH for increased growth of fungal mycelium, pigment production in the Potato Dextrose Broth is pH 4. According to the report, different types of fungal strains have more acidic pH during submerged fermentation for pigment production[3]. Fungal biomass was filtered and washed in the deionized water and dried at 50º C and weighted and graph, where plotted accordingly in Figure **7**. Moreover, according to the study done by[4], notified that maximum fungal biomass and bioactive pigment yield were obtained at 28 °C and pH 5.0. Consequently, our studies revealed that the maximum biomass and pigment yield of the selected strain was obtained at 25ºC and pH 4.0

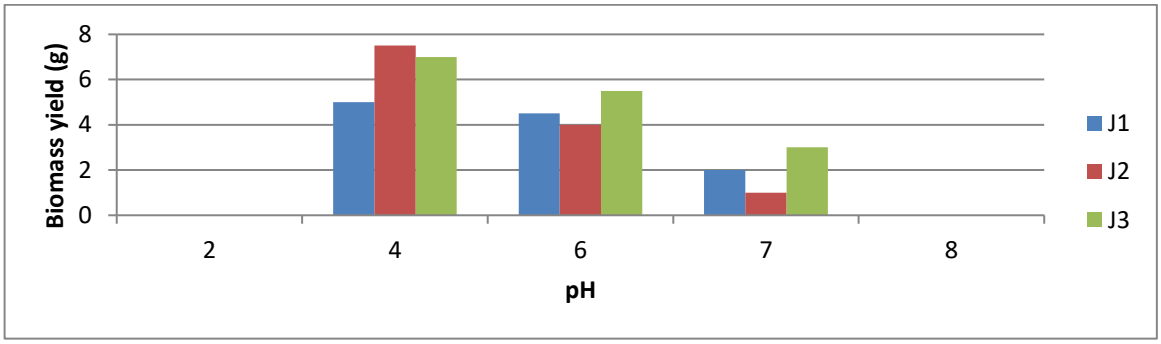


Figure 7 Effect of different pH on J1, J2, and J3, where the x-axis represents the initial ph of the fungal medium and the y-axis represents the cell dry weight (grams) after the 5 days of incubation.

3.3.5 Effect of temperature**:**

Besides the optimum temperature studied by the fungal mycelium and pigment production in Potato Dextrose Broth in shake flask at different temperatures (20, 25, 30, 35, 40) it is found to be 25°C was the optimum temperature to increase the growth of pigment production. Therefore, isolated culture can optimally grow at 25°C temperature. Fungal biomass was filtered and washed in the deionized water and dried at 50ºC and weighed and the graph was plotted accordingly in Figure **8**. Moreover, the study done by[4] said that the maximum organisms' biomass and pigment production were obtained at 28 °C and pH 5.0. Consequently, our studies revealed that a large quantity of biomass and pigment yield of selected strain was accomplished at 25ºC and pH 4.0

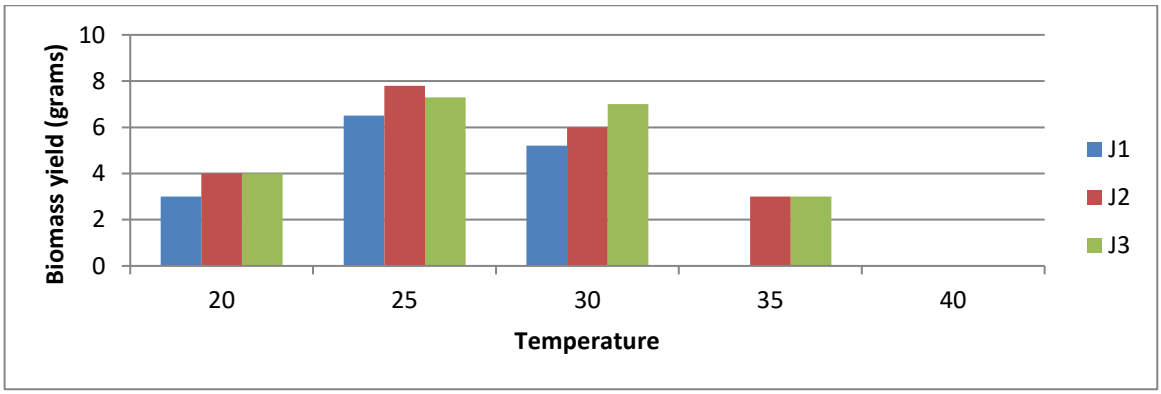


Figure 8 Effect of different temperature on J1, J2, and J3, where the x-axis represents the initial temperature (degree Celsius) of the fungal medium and y axis represent the cell dry weight at the grams after the 5th days of incubation.

3.4 Production of fungal pigments by submerged fermentation

Production of extracellular pigments from isolated fungal strains is carried out on the basis of optimization. The pigment production was observed on the 7th day at pH-4 at 25ºC by the submerged fermentation shown in (Figure. **9**). The isolated strains J1, J2, and J3 showed no consequential difference in mycelium dry weight after 5th days of the incubation period. However, an increase in pigment production was observed up to the 7th day of incubation. The results obtained were found to correlate with the research studies done by[4] whereas, [18]has published studies which shows maximum fungal biomass and pigment production within 4 days of the latent period. Therefore, the latent

period of fungal strains for pigment production of 7 days was selected as the optimum time period based on the present investigation and previous literature manifest to accomplish a maximum yield[19].

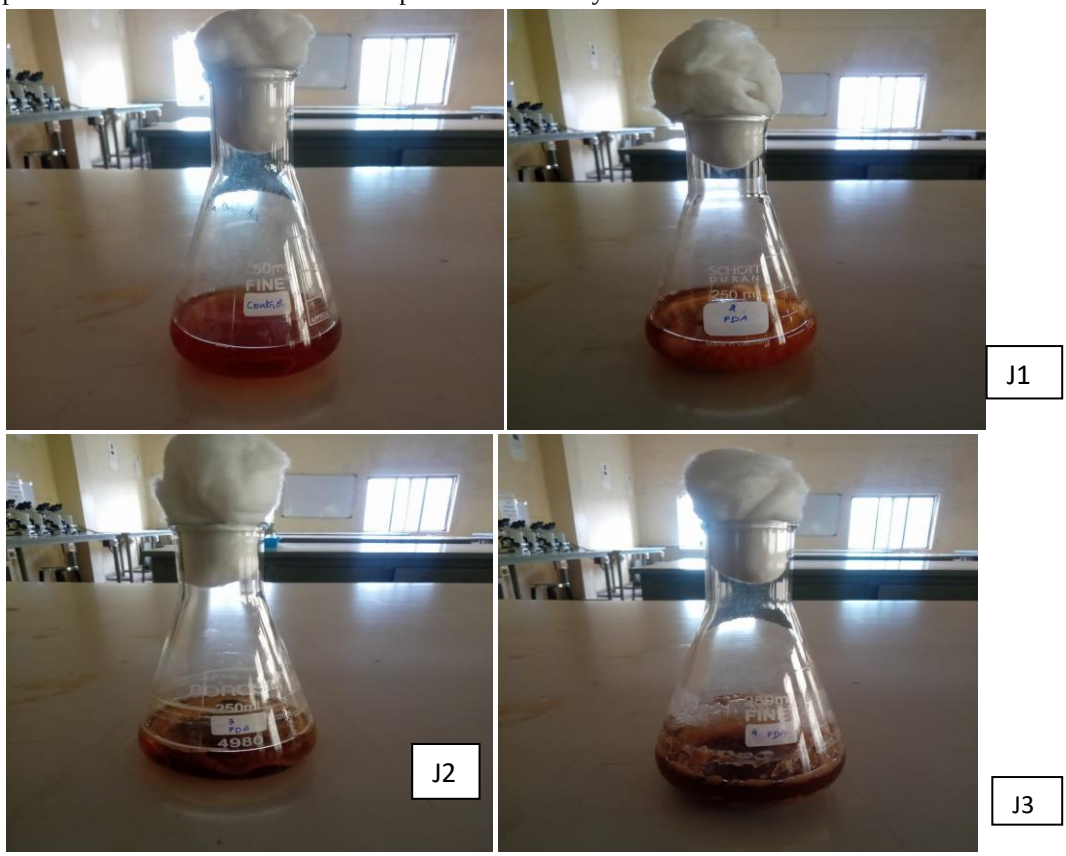


Figure 9 Production of the pigment carried in the optimized media by submerged fermentation in the shaking incubator for 7 days.

3.5 Extraction of the fungal pigments

3.5.1 Solubility of the pigments

The extracted pigment is checked for solubility in organic solvents and water and tabulated (table **3**). The isolated strains J1, J2, and J3 are soluble in water and also soluble in an organic solvent in acetone. As Acetone is used as the best solvent extract therefore it conforms to the solubility of the pigment.

FUNGAL STRAINS	HEXANE	PETROLEUM ETHER	BUTANOL	ACETONE	CHLOROFORM	ETHYL ACETATE
J1	-	-	-	+	-	-
J2	-	-	-	+	-	-
J3	-	-	_	+	-	-

Table 3 Solubility of fungal compounds extracted with various solvents

3.6 Characterization of fungal pigments

3.6.1 Column chromatography

The crude pigment was fractionated and collected the 15 fractions adsorbed to the silica slurry in the column were then eluted with acetic acid: ethyl ether (1:10). Then, the Thin Layer Chromatography was performed to determine the compound in the different fractions.

3.6.2 Thin layer chromatography

In this study, thin-layer chromatography was used to assess the underlying pigments produced by each fungal isolates. The results presented in Figure. **10**, indicate that the pigments from different microfungi have multi-component in nature. A thin layer chromatographic method was performed and the R_f value of the black, brown, and orange pigments was found to be 0.4 and 0.66. According to the research work, of Mac Faddin et al the R_f value was found to be 0.6 and they were found as a carotenoid.

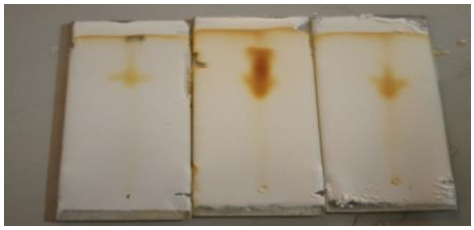


Figure 10 Thin layer chromatography of the pigment compound of the A) J1, B) J2, C) J3 fungal isolates when their retention factor was calculated as A) R_f= 0.6 B) R_f=0.4 C) R_f=0.4

3.7 Antibacterial activity of fungal pigments

3.7.1 Minimum inhibitory concentration method

The Minimum Inhibitory Concentration (MIC) determined is performed to check the concentration at which crude extracts inhibit the test pathogens. The MIC of the J2 and J3 strains are only active potentials against the pathogens since it is high purity compared to J1. MIC concentration was determined by inhibition growth of the test pathogen that is checked by its turbid. The MIC concentration of the J2 and J3 against the two test pathogens is 40 mg/ml. And for J1 they didn't show any activity against pathogens as it shows no turbidity.

3.7.2 Agar well diffusion method:

The extracted biologically active pigment from fungal strains was subjected to an antibiotic sensitivity test. In the screening of antibacterial ability, the orange pigment showed no activity this result linked with Kim et al. (2006) that, Monascus orange pigment reported little or no activity, while the other green pigment showed a broad spectrum of antibacterial effect against Pseudomonas aeruginosa and the maximum inhibition zone is 32 mm and minimum inhibition zone is 22.5 mm as shown in (Table **4**). The isolate J1 shows no effect against Bacillus cereus and Pseudomonas aeruginosa. As referred D. Saravanan et al their isolate Aspergillus sp., (Black, Brown) shows no antibacterial activity against the test pathogen Bacillus cereus and Pseudomonas aeruginosa but to the isolate J2, J3 strain shows antibacterial activity (Figure **11a**, **11b**).

STRAIN	CONCENTRATION (µL)	ZONE OF INHIBITION (mm)	
		Bacillus cereus	Pseudomonas aeruginosa
J1	25	-	-
	50	-	-
	75	-	-
	100	-	-
J2	25	-	-
	50	-	-
	75	20	22
	100	30	28
J3	25	-	-
	50	-	-
	75	28	32
	100	38	40

Table 4 Antibacterial activity of fungal strains J1, J2, J3 by Agar well diffusion (Kirby bauer) method against the test pathogens of Gram-positive bacteria Bacillus cereus., and Gram-negative bacteria Pseudomonas aeruginosa. The zone of inhibition was measured in the zone meter scale (mm).

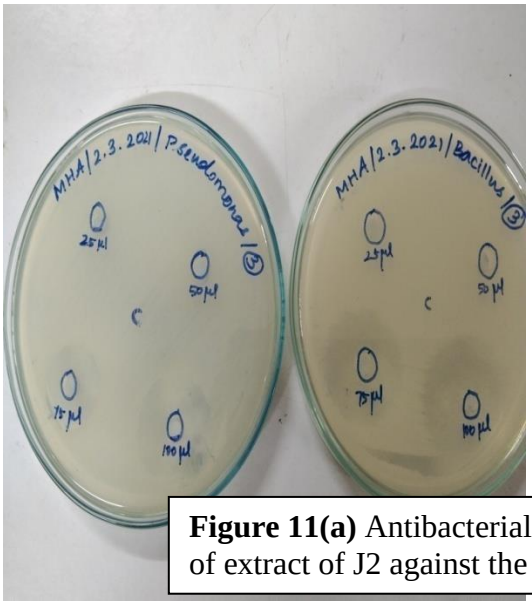


Figure 11(a) Antibacterial activity of extract of J2 against the test

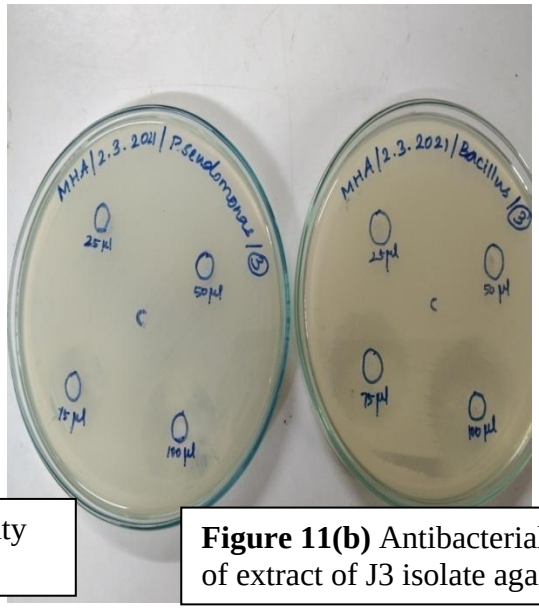


Figure 11(b) Antibacterial activity of extract of J3 isolate against the

3.8 Effectiveness of pigment in candle

The pigments extracted from the J1 isolate were used as an application for dyeing the candle. The plain white colored soap was dyed orange with the pigments of J1 isolate. The colour doped in the candle was more effectively neither than cloth.

A B

Figure 12

A) Represent the mold before the addition of pigment (J1)

B) Represents the mold after the addition of the pigment (J1)

3.9 Effectiveness of pigment in cloth

The pigments extracted from the J1 isolate were used as an application for dyeing the cloth. The plain white colored cloth was dyed orange with the pigments of J1 isolate. The colour doped in the cloth was initially orange but after wash slight change in colour.

A B

Figureure 13

A) Represent the cotton cloth without coating the pigment suspension of J1

B) Represent the cotton cloth mordant by dying of pigment suspension of J1.

4 CONCLUSIONS

Pigments have great importance in food colorants, cosmetics, etc. Microorganism like bacteria, fungi, Actinomycetes, and archeae among these fungi is an interesting source of pigment production. Recent publications showed that around 47% of papers on fungi are capable of producing pigments. Microorganisms are less/unexplored ecosystems as it is a promising source for novel biomolecules.

From this preliminary study, it is concluded that fungal strains obtained from garbage dump soil synthesize the pigments. After selecting these three fungal strains, optimization has been done in which the effect of carbon source, nitrogen source, pH, and temperature has been done. The optimization studies, reveal that the maximum biomass has high pigment production. The maximum biomass growth of fungi in particular nutrient sources has a maximum optical density (extracted crude extract). The negative results showed in antibacterial effect of orange pigment J1 showed that they can be used for human consumption as they do not interfere with the human biota but further toxicity is needed to confirm its property and also can have a potential application as food color additive and positive result in antibacterial effect of green and black pigment of J2, J3 showed that they can be used in pharmacological applications. Thus, we conclude that pigments can be used for food additives and color which leads to developing new sources of bio-colors which can be easily cultivated from the fungal strains that can be further explored at larger scale production on an industrial scale. Further studies of identification pigment by analytical methods and their toxicity, and washing durability are done for future studies.

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