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Improving Productivity of Bio Melanin Pigment Extracted from local Aspergillus Niger Isolate from Contaminated Dry Food Using Uv Method Mutagenesis

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ABSTRACT: This study explored the use of UV-C irradiation as a physical mutagen to enhance melanin biosynthesis in Aspergillus niger isolates.. Twenty-three fresh and dried food samples were collected, yielding twelve isolates of Aspergillus niger. Morphological identity was confirmed by amplifying the internal transcribed interstitial region (ITS) using polymerase chain reaction (PCR) with the generic primers ITS1–ITS4. Amplification was confirmed in 90% of the studied isolates. Melanin was extracted from the selected isolate and characterized using UV-Vis spectroscopy and Fourier transform infrared (FTIR) analysis. The native strain produced approximately 23 mg/g of melanin of biomass. Genetic modification via mutagenesis was performed using UV-C (254 nm) irradiation at exposure distances of 5, 10, 15, 20, and 25 cm, and for durations of 30, 90, 180, 360, 540, and 720 seconds, respectively. The highest melanin yield (48 mg/g biomass) was obtained after irradiation for 360 seconds at a distance of 15 cm. These results demonstrate that UV-controlled mutagenesis is an efficient and economical approach for enhancing melanin production in Aspergillus niger.

1-INTRODUCTION:

Microorganisms are a source of metabolites of significant industrial value, including enzymes, pigments, organic acids, and other bioactive compounds. complex genetic and metabolic regulatory networks under natural conditions determine metabolite production. Although improved breeding methods enhance productivity, these biological processes are highly influenced by genetic factors. Consequently, strains and mutations are frequently improved to increase productivity for economic exploitation. (Nielsen & Keasling, 2016)

One of the most important microorganisms used in biotechnology is the fungus *Aspergillus niger*, specifically the Aspergillus nigri subspecies. This filamentous fungus is widely distributed in natural and agricultural environments. This isolate is widely used in industrial production, particularly for the production of citric acid, enzymes, and numerous value-added metabolites, due to its metabolic diversity and high secretory capacity. (Cairns et al., 2018).

Melanin, a high molecular weight pigment produced by microorganisms, especially fungi, and sometimes by plants or animals, has become the subject of widespread scientific interest and a focus of attention in medicine, biotechnology, space technology, and environmental remediation. It possesses unique physical and chemical properties and has numerous biological functions, such as resistance to harsh environmental conditions and oxidative stress, and protection against ultraviolet radiation.. (Tran-Ly et al., 2020).

One type of filamentous mold, *Aspergillus niger,* has been shown in numerous studies and research to be highly active in industrial fermentation processes and to have a high tolerance for harsh conditions, emerging as an important biological source for melanin production. (Koch et al., 2023).

Melanin pigment is composed of a disorganized group of complex biopolymers, which vary according to their biosynthetic pathways and constituent molecules. The main classes include eumelanin, pheomelanin, pyomelanin, allomelanin, and neuromelanin. In fungi, melanin contributes significantly to cellular defense by reducing the harmful effects of ultraviolet radiation and reactive oxygen species through free-radical scavenging and protection of intracellular macromolecules, particularly DNA (Tran-Ly et al., 2020).

Genetic variation is a fundamental component of biological evolution and adaptation. One of the primary sources of such variation is mutation, which is defined as a heritable alteration in the nucleotide sequence of DNA. Mutations may occur spontaneously or be induced by physical, chemical, or biological mutagens. Consequently, mutagenesis has become an important tool in microbial strain improvement programs aimed at enhancing the production of commercially valuable metabolites (Krašovec et al., 2021).

Ultraviolet (UV) radiation is among the most widely used physical mutagens in microbial biotechnology. Exposure to UV light can induce DNA damage through the formation of pyrimidine dimers, leading to genetic alterations that may affect cellular metabolism and gene regulation. Several studies have demonstrated that UV-induced mutagenesis can improve pigment biosynthesis in fungi by modifying regulatory pathways and increasing the expression of genes involved in pigment formation (Koch, S. M., et al., 2023).

The present study aimed to enhance melanin production by *Aspergillus niger* through ultraviolet-induced mutagenesis. Food samples exhibiting fungal contamination were collected and used for fungal isolation. Preliminary screening, microscopic identification, molecular confirmation using PCR, pigment extraction, FTIR analysis, UV–Visible spectroscopy, and UV mutagenesis were subsequently performed to evaluate their effects on melanin production.

2- MATERIALS AND METHODS

2-1 Chemicals and media

Culture media was used potato dextrose agar ,Rose Bengal agar , malt extract agar, potato dextrose broth . Chemical material is Athelacetate ethanol lactophenol cotten blue, agarose, ethidium bromid, buffer solution, NaOH (PH:13), (2M) HCl , (6M) HCl ,(TBE) ,EDTA , KBr.

2.2 Sample Collection

A total of twenty-three food samples, including fresh and dried food products, were collected from different local markets in Baghdad, Iraq, between January and April 2026. The collected samples were aseptically transferred to the laboratory and processed immediately for fungal isolation to minimize contamination and preserve the viability of fungal propagules.

2.3 Isolation and Identification of Fungi

Fungal isolation was performed using the serial dilution technique following the procedure described by Waksman (1972). Briefly, appropriate serial dilutions were prepared from each sample suspension, and aliquots were inoculated onto Potato Dextrose Agar (PDA), Rose Bengal Agar (RBA), and Malt Extract Agar (MEA). The inoculated dishes were incubated at a temperature of 25-28°C and humidity until distinct fungal colonies grew.

A total of twelve fungal isolates were recovered from the collected food samples. Preliminary identification of the isolates was carried out according to the taxonomic criteria described by Pitt and Hocking (1997). Among the recovered isolates, *Aspergillus niger* was selected for further investigation because of its potential ability to produce melanin pigment. The selected isolate was purified by repeated subculturing on Potato Dextrose Agar (PDA) to obtain a single, pure culture.

Morphological identification of *Aspergillus niger* was based on both macroscopic and microscopic characteristics. . Domsch and Gams (1988). Colony morphology, colour, texture, growth rate, and pigmentation were examined macroscopically, whereas microscopic identification included observations of conidiophores, vesicles, phialides, metulae, and conidia The fungal isolate was identified using a polyphasic approach based on macroscopic, microscopic, and molecular characteristics according to Houbraken

et al. (2020) and Samson et al. (2024) The morphological identification was subsequently confirmed by molecular analysis using polymerase chain reaction (PCR)

2.4 Molecular Identification of *Aspergillus niger* by Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from ten *Aspergillus niger* isolates using the HiPurA™ HP Fungal DNA Purification Kit (HiMedia Laboratories Pvt. Ltd., India) according to the manufacturer's protocol.

Prior to DNA extraction, the fungal isolates were cultivated in Potato Dextrose Broth (PDB) and incubated at 25–28°C for five days in a rotary shaker at 30 rpm to obtain sufficient fungal biomass. The fungal mycelia were harvested by centrifugation, the supernatant was discarded, and the pellets were air-dried before DNA extraction.

The presence and concentration of 1.5% genomic DNA prepared in a 1× Tris-Borate-EDTA (TBE) buffer containing ethidium bromide were confirmed by electrophoresis on an agarose gel. A 1× agarose gel was prepared by dissolving 1.5 g of agarose in 100 mL of TBE buffer, followed by the addition of 5 µL of ethidium bromide. After complete dissolution, the gel was poured into a tray, allowed to solidify, and then immersed in the electrophoresis solution.. DNA samples were placed in the gel wells and subjected to electrophoresis at 80 V for approximately 60 minutes. The DNA bands were imaged using ultraviolet light. (Sambrook & Russell, 2001).

Molecular identification of the selected fungal isolates was performed by amplification of the Internal Transcribed Spacer (ITS) region using the universal fungal primer pair ITS1 and ITS4, which is considered the standard DNA barcode for fungal identification (White et al., 1990; Schoch et al., 2012).

The nucleotide sequences of the primers used in this study were as follows:

ITS1: 5'–TCCGTAGGTGAACCTGCGG–3'

ITS4: 5'–TCCTCCGCTTATTGATATGC–3'

PCR amplification was carried out using reagents supplied with the HiPurA™ PCR Kit (HiMedia, India). The reaction mixture was prepared in a final volume of 25 µL, as presented in Table (1).

Table (1). Composition of the PCR reaction mixture

size	chemical (Volume µL)
12.5	2X PCR master mix solution
2	Reverse primer
8.5	Nuclease-free Water
2	DNA
25	Total

PCR amplification was performed in a thermal cycler using the following cycling conditions: an initial denaturation step at 95°C for 3 min, followed by 35 amplification cycles, each consisting of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 1 min. A final extension step was carried out at 72°C for 7 min, after which the reactions were held at 4°C until analysis (White et al., 1990).

The amplified PCR products were separated by electrophoresis on 1.5% agarose gel in 1× TBE buffer at 80 V for approximately 60 min. using an ultraviolet light source, DNA fragments were observed and imaged to verify the presence of the expected ITS amplification, to confirm the molecular identification of the selected *Aspergillus niger* isolates. (Schoch et al., 2012; Raja et al., 2017).

2.4 Preparation of Fungal Spore Suspension

To prepare the spore suspension, a seven-day-old pure culture of *Aspergillus niger* grown on potato and dextrose agar (PDA) was used. Five milliliters of sterile distilled water containing 0.01% (v/v) Tween 80 were aseptically added to each culture. Using a

sterile inoculation ring, the surface of the fungal colony was gently scraped to release the conidia into the suspension. The resulting suspension was shaken on a rotary shaker for 30 minutes to obtain a homogeneous spore suspension and separate the aggregated conidia. The suspension was then filtered through sterile cotton to remove the fungal hyphae and obtain a pure conidia suspension. (Samson et al., 2019).

Using a multi-chambered blood cell counter under a light microscope, the concentration of fungal spores was determined. A small amount of spore suspension was placed in the counting chamber, and according to the standard blood cell counter equation, the spores were counted in the designated squares, and the spore concentration was calculated. (CLSI,2022):

Spore concentration (spores mL^{-1}) = (Average number of spores counted × Dilution factor × 10^4)

The spore suspension was then adjusted to the desired concentration for subsequent mutagenesis experiments.

2.5 Extraction of Melanin Pigment

Using an alkaline extraction method with modifications based on previously published protocols (Tran-Ly et al., 2020; Koch et al., 2023), melanin pigment was extracted from *Aspergillus niger* isolates. Fungal biomass was collected from the culture medium by centrifugation and thoroughly washed with distilled water to remove any residual medium components. The collected biomass was suspended in a 2 M sodium hydroxide (NaOH) solution at a pH of 12.5. The suspension was then heated to facilitate melanin solubility and incubated for 36 hours.

The suspension was then centrifuged at 4000 rpm for 15 minutes, and the filtrate containing the dissolved melanin was collected. Melanin was precipitated using 2 M hydrochloric acid (HCl), adjusting the pH of the filtrate to 2.5 and incubating it at room temperature for 2 hours. The precipitated pigment was collected by centrifugation at 4000 rpm for 15 minutes.

The precipitated melanin was then treated with 6 M hydrochloric acid at 100°C for 2 hours to remove contaminating proteins, carbohydrates, and other cellular components.The purified dye was then dissolved in 2 M sodium hydroxide, and centrifuged again to remove any undissolved impurities. The supernatant containing the purified melanin was collected. The dye was then dried in a hot air oven at 60°C and stored in sterile containers for analysis.

2-6 UV – Visible Spectroscopy:

The extracted melanin pigment was characterized using a UV–Visible spectrophotometer (Shimadzu UV-1800, Japan). The pigment sample was dissolved in 2 M NaOH to obtain a homogeneous solution, and the absorbance spectrum was recorded using a quartz cuvette with a 1 cm optical path length. The absorption spectrum was measured over a wavelength range of 200–800 nm, using 2 M NaOH as the blank solution. The absorbance values were recorded automatically, and the relationship between absorbance (A) and wavelength (λ) was plotted to determine the characteristic absorption profile of the extracted melanin pigment. , which is considered one of the characteristic spectral features of melanin pigments (Tran-Ly et al., 2020; Koch et al., 2023).

2.7 Fourier Transform Infrared (FTIR) Spectroscopic Analysis

The functional groups present in the extracted melanin pigment were characterized using Fourier Transform Infrared (FTIR) spectroscopy. Approximately 1 mg of the dried melanin sample was thoroughly mixed with 100 mg of spectroscopic-grade potassium bromide (KBr). The mixture was finely ground using an agate mortar and pestle to obtain a homogeneous powder. Subsequently, the mixture was compressed into a transparent pellet using a hydraulic press under a pressure of 10 tons for 5 min.

The prepared KBr pellet was analyzed using an FTIR spectrometer, and spectra were recorded over the spectral range of 400–4000 cm^{-1}. The obtained spectra were used to identify the characteristic functional groups of the extracted melanin pigment by analyzing the corresponding absorption bands . (Bayram, S. 2022) , Singh, S et al.,(2021)

2.8 Ultraviolet (UV) Mutagenesis

Physical mutagenesis was performed using ultraviolet (UV-C) radiation at a wavelength of 254 nm to generate mutant strains of *Aspergillus niger* At exposure distances of 5, 10, 15, 20, and 25 cm, for 30 seconds, 90 seconds, 3, 6, 9, and 12 minutes respectively, a freshly prepared suspension of conidia, containing approximately 1 × 10^6 spores/ml, was exposed to UV radiation. All

irradiation procedures were carried out under dark conditions with stirring to prevent photoreactivation and ensure uniform UV exposure of the spores. (Leavell, et al., 2020).

After irradiation, 0.1 ml of each treated spore suspension was spread on potato dextrose agar (PDA) plates, along with the untreated control sample, and incubated at 28°C for 3-5 days. By comparing the number of developing colonies obtained after UV treatment, the survival rate of the irradiated spores was determined with the number of developing colonies in the untreated control group.

Isolated and mutant colonies exhibiting a survival rate of less than 1% were considered acceptable for testing, as lower survival rates generally indicate higher mutational efficiency (Chen et al., 2020). Seven mutant colonies obtained after 6 minutes of UV exposure at a distance of 15 cm were selected for melanin pigment extraction. The selected mutant isolates were recultured for three successive generations to assess their genetic stability before being introduced into melanin production. Stable mutant strains exhibiting enhanced pigment production were then selected.

3-RESULT & DISCUSSION

3.1 Isolation and Identification of Fungi

Mycological examination of the 23 fresh and dried food samples collected from different markets in Baghdad Governorate revealed a diverse fungal population. The isolated fungi belonged to the genera *Penicillium, Aspergillus, Trichoderma, Emericella, Cladosporium, Fusarium, Aureobasidium, Alternaria,* and *Rhizopus.* Twelve isolates were identified as *Aspergillus niger* according to their morphological characteristics and were selected for subsequent analyses. (Samson, R et al 2019).

Aspergillus niger is one of the most common and widespread fungi isolated from dry stored materials. It adapts to environments with low water activity and high osmotic pressure. The deposition of melanin in its cell walls allows it to withstand environmental stresses such as ultraviolet radiation, dehydration, and oxidative damage. This is due to the dark pigmentation of the spores caused by melanin, which is enhanced during prolonged food storage under these conditions, as confirmed by studies (Pitt, J. I., & Hocking, A. D. 2022).

3.2 Molecular Identification of *Aspergillus niger*

By performing molecular analysis using polymerase chain reaction (PCR) to amplify the transcriptionated inner interval (ITS) region of RNA using the generic primers ITS1 and ITS4, the isolate of *Aspergillus niger* was confirmed. The ITS region is a standard molecular marker for fungal identification due to its high species-discrimination capacity. After analyzing ten putative *Aspergillus niger* isolates, successful amplification of the predicted PCR product (approximately 599 base pairs) was confirmed in nine isolates, representing a 90% confirmation rate. (Figure 1). The amplified fragments corresponded to the expected size of the ITS region, confirming the identity of the selected isolates as *A. niger*. These findings demonstrate that PCR-based identification provides a rapid and reliable approach for confirming morphological identification of Aspergillus species. (Schoch et al., 2012)

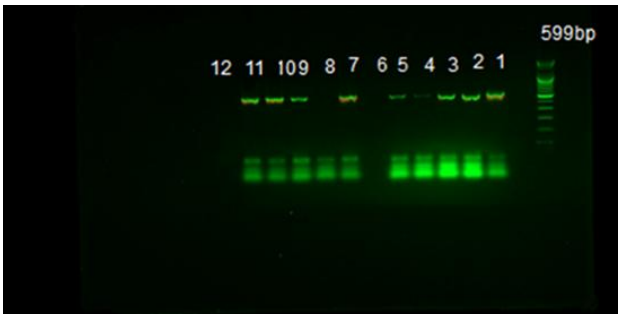

Figure 1. PCR amplification of the ITS region of *Aspergillus niger* isolates (599 bp).

The molecular identification results confirmed the morphological diagnosis of *Aspergillus niger*, with successful amplification of the expected ITS fragment (≈599 bp) in 90% of the examined isolates. The ITS region is considered the universal molecular marker for accurate fungal identification at the species level. Similar findings have been reported in previous studies that demonstrated the high reliability of ITS-based PCR for the identification of *Aspergillus species;(Raja et al., 2017).

3.3 Extraction of Melanin Pigment

The melanin pigment was successfully extracted from the selected dark-pigmented *Aspergillus niger* isolate using the alkaline-acid extraction method. Melanin was identified by its strong absorption in the ultraviolet region with a gradual decrease in absorbance toward the visible region Approximately 23 mg of melanin per gram of fungal biomass was obtained. The extracted pigment was subsequently characterized by several analytical techniques, including UV–Visible spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy, to confirm its identity as melanin .

The successful recovery of melanin from *Aspergillus niger* demonstrates the ability of this fungus to synthesize dark extracellular pigments under suitable growth conditions. The conditions of development, methods of extraction, and type of isolation have an effect on the extraction yield, and research has shown that the yield of the dye varies according to the types of Aspergillus fungi that produce melanin. (Tran-Ly et al., 2020).

3.3 UV–Visible Spectroscopy

The UV-Vis absorption spectrum of the extracted melanin pigment confirmed the characteristic optical behavior of melanin, exhibiting a strong absorption peak at 215 nm and a second, broader peak at approximately 304 nm. The absorption gradually decreased with increasing wavelength towards the visible region, demonstrating the typical steady-state decrease associated with melanin pigments (Figure 2). A linear relationship with a negative slope was observed in the logarithmic graph of absorption versus wavelength (400–600 nm), further confirming the distinctive optical properties of melanin.

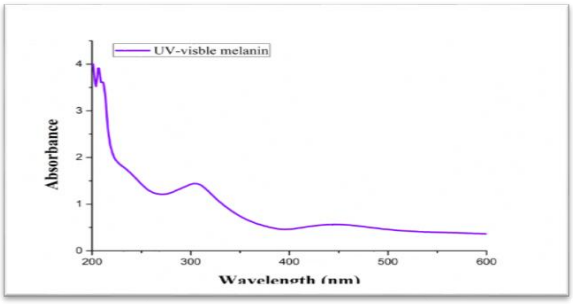

Figure 2. UV–Visible absorption spectrum of melanin extracted from *Aspergillus niger*

The spectrum shown in the figure is characteristic of innate melanin, suggesting a heterogeneous aromatic polymer structure that allows for efficient absorption of ultraviolet radiation across a wide range of wavelengths. A common and distinctive feature of biogenic melanin is the gradual decrease in absorption towards the visible light region.Similar absorption patterns in the ultraviolet and visible ranges have been described by... Suwannarach et al. (2019), Seelam et al. (2021), Elsayis et al. (2022), and Tran-Ly et al. (2020).

3.4 Fourier Transform Infrared (FTIR) Analysis

The FTIR spectrum of the extracted pigment exhibited several characteristic absorption bands confirming its identity as melanin (Figure 3). A broad absorption band observed within the 3400–2900 cm^{-1} region was assigned to O–H and N–H stretching vibrations, indicating the presence of hydroxyl and amino functional groups. Characteristic absorption bands at 1600, 1456, 1203, 848, and 576 cm^{-1} were attributed to aromatic C=C stretching, aliphatic C–H bending, C–O stretching, aromatic C–H out-of-plane bending, and aromatic skeletal vibrations, respectively. These functional groups are characteristic of fungal melanin and confirm the complex aromatic polymeric structure of the extracted pigment.

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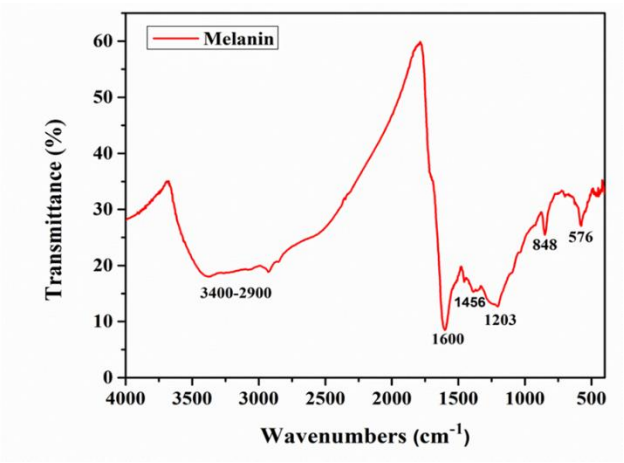

Figure 3: Fourier transform infrared (FTIR) spectrum of melanin extracted from *Aspergillus niger*.

The FTIR spectrum obtained in the present study agrees with the characteristic structural features of fungal melanin reported in previous studies. The presence of hydroxyl, amino, aromatic, and phenolic functional groups confirms the heterogeneous polymeric nature of melanin, which is responsible for its antioxidant, metal-chelating, and photoprotective properties. Similar FTIR profiles have been reported for fungal melanin extracted from Aspergillus species and other melanized fungi (Pralea et al., 2019; Tran-Ly et al., 2020).

3.5 Effect of UV Mutagenesis on *Aspergillus niger*

The effect of different UV-C irradiation doses (254 nm) on the survival of *Aspergillus niger* spores is presented in Table 2. With increasing exposure time and decreasing irradiation distance, a gradual decrease in the survival rate of spores is observed compared to the untreated control sample, indicating the dependence of a dose that has an inhibitory effect of ultraviolet radiation. When the doses of irradiation are increased, a complete loss of viability is observed, while a limited survival rate is recorded under moderate exposure conditions..

Table (2) effect the doses of UV ray in Asp.niger isolate (colony) for several times (seconds) and distances(centimeter)

	5 cm	10 cm	15 cm	20 cm	25 cm	control
30 s.	6 c.	81 c.	89 c.	92 c.	88 c.	147 c.
90 s.	21 c.	21 c.	17 c.	25 c.	65 c.	
180 s.	16 c.	23 c.	24 c.	29 c.	62 c.	
360 s.	2 c.	3 c.	7 c.	15 c.	34 c.	
540 s.	-	-	1 c.	1 c.	3 c.	
720 s.	-	-	-	1 c.	-	

cm: centimeter, s: seconds , c: colony

Among the tested treatments, irradiation for 360 s at a distance of 15 cm produced the most efficient mutant, yielding approximately 48 µg of melanin g^{-1} biomass, compared with 23 µg g^{-1} biomass produced by the parental (control) strain (Figure 4). Higher or shorter exposure times did not result in good melanin production, indicating that an optimal dose of ultraviolet radiation is required to stimulate beneficial mutations without causing excessive cell damage.

The DNA damage caused by excessive UV-C exposure is attributed to the reduced spore survival rate after UV irradiation. The formation of cyclic pyrimidine dimers (CPDs) and other photosynthetic byproducts that inhibit DNA replication and transcription causes damage, leading to persistent cellular deterioration and loss of viability. However, the induction of beneficial mutations that promote the biosynthesis of secondary metabolites can occur during moderate UV exposure. UV-induced

mutations may have altered genes involved in melanin biosynthesis or their regulatory pathways, resulting in the increased melanin production observed in the selected mutant. This finding supports the results of studies that have improved microbial strains using UV-induced mutations. (Kodym & Afza, 2003; Adrio & Demain, 2014).

3.6 Effect of UV Mutagenesis on Melanin Production

The irradiation times and distances used were adopted. Irradiation of *Aspergillus niger* spores for 360 seconds at a distance of 15 cm resulted in high melanin production, reaching approximately 48 mg/g of biomass, compared to 23 mg/g of biomass in the untreated control group. (Figure *4*). In contrast, irradiation at other distances and time periods led to decreased melanin production, indicating that the UV dose strongly influences melanin biosynthesis. These results suggest that an optimal level of UV-induced mutations can enhance pigment production without causing excessive cell damage..

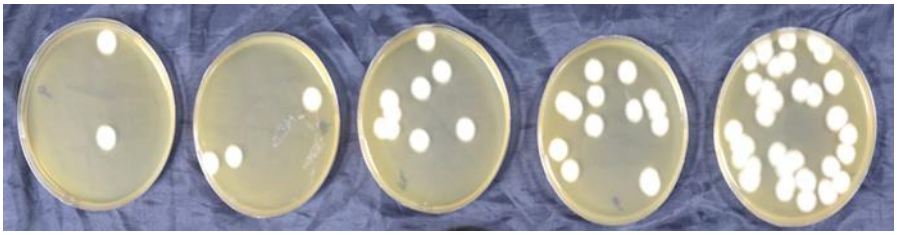

Figure 4: Effect of UV ray exposure on colonies of *Asp.niger* isolates in culture media

The increased melanin production observed after exposure to an optimal dose of ultraviolet (UV) radiation is likely linked to mutations affecting genes involved in melanin biosynthesis or their regulatory mechanisms. These genes are likely affected after exposure to an optimal dose of radiation, leading to increased melanin production. UV radiation is known to induce the formation of cyclic pyrimidine dimers (CPDs) and other DNA lesions that generate genetic diversity, which may give rise to mutations with enhanced metabolic capabilities. Researchers have reported similar improvements in the production of microbial metabolites after UV exposure in previous studies on improving fungal strains. (Adrio & Demain, 2014; Nielsen, & Keasling, 2022).).

4- CONCLUSION

From fresh and dried food samples, the fungus *Aspergillus niger* was isolated and its identity was definitively established through morphological characteristics and molecular analysis. It produced 23 mg of melanin per gram of biomass. UV-Vis and FTIR analyses confirmed that the extracted pigment was melanin. UV-C induced mutagenesis effectively enhanced melanin biosynthesis, with optimal treatment (360 seconds at a distance of 15 cm) increasing pigment production to approximately 48 mg/g of biomass, representing an improvement in pigment yield compared to the native strain. One of the effective and economical methods for improving melanin productivity from Aspergillus isolate is UV-induced mutagenesis, as proven by research results, highlighting its potential for future biotechnological and industrial applications.

5- RECOMMENDATION

Conducting studies on other fungal species to verify the production and characterization of melanin pigments with important and useful industrial and medical applications, as well as linking them to nanotechnology systems and developing antioxidants and UV protection materials.

RREFERENCES:

[1] Adrio, J. L., & Demain, A. L. (2014). Microbial strain improvement for production of metabolites and enzymes. FEMS Microbiology Reviews, 38(1), 19–40. https://doi.org/10.1111/1574-6976.12073

[2] Bayram, S. (2022). Comparative characterization study between fungal and bacterial eumelanin pigments. Indian Journal of Microbiology, 62(3), 393–400. https://doi.org/10.1007/s12088-022-01012-1

[3] Cairns, T. C., Nai, C., & Meyer, V. (2018). How a fungus shapes biotechnology: 100 years of Aspergillus niger research. Fungal Biology and Biotechnology, 5, 13. https://doi.org/10.1186/s40694-018-0054-5

[4] Chen, L., Xin, Q.-H., Ma, L.-M., Li, R.-F., & Bian, K. (2020). Applications and research advance of genome shuffling for industrial microbial strains improvement. World Journal of Microbiology and Biotechnology, 36, 158. https://doi.org/10.1007/s11274-020-02936-w

[5] Clinical and Laboratory Standards Institute (CLSI). (2022). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi (M38, 3rd ed.). CLSI.

[6] Elsayis, A., Hassan, S. W. M., Ghanem, K. M., & Khairy, H. (2022). Optimization of melanin pigment production from the halotolerant black yeast Hortaea werneckii AS1 isolated from a solar saltern in Alexandria. BMC Microbiology, 22, 232.

[7] Houbraken, J., Frisvad, J. C., & Samson, R. A. (2020). Taxonomy of Penicillium and Talaromyces and their relationships with Aspergillus. Studies in Mycology.

[8] Koch, S. M., Freidank-Pohl, C., Siontas, O., & Meyer, V. (2023). Aspergillus niger as a cell factory for the production of pyomelanin, a molecule with UV-C radiation shielding activity. Frontiers in Microbiology, 14, 1233740. https://doi.org/10.3389/fmicb.2023.1233740

[9] Kodym, A., & Afza, R. (2003). Physical and chemical mutagenesis. In Plant Functional Genomics (pp. 189–203). Humana Press. https://doi.org/10.1385/1-59259-413-1:189

[10] Krašovec, R., et al. (2021). Spontaneous mutation rate is a plastic trait associated with population density across domains of life. PLoS Biology, 19(8), e3001454. https://doi.org/10.1371/journal.pbio.3001454

[11] Leavell, M. D., Singh, A. H., & Kaufmann-Malaga, B. B. (2020). High-throughput screening for improved microbial cell factories: Perspective and promise. Current Opinion in Biotechnology, 62, 22–28. https://doi.org/10.1016/j.copbio.2019.07.002

[12] Nielsen, J., & Keasling, J. D. (2016). Engineering cellular metabolism. Cell, 164(6), 1185–1197. https://doi.org/10.1016/j.cell.2016.02.004

[13] Nielsen, J., & Keasling, J. D. (2022). Engineering cellular metabolism for the production of valuable chemicals. Cell, 185(5), 881–897. https://doi.org/10.1016/j.cell.2022.02.004

[14] Pitt, J. I., & Hocking, A. D. (2022). Fungi and food spoilage (4th ed.). Springer Nature. https://doi.org/10.1007/978-3-030-82606-8

[15] Pralea, I. E., Moldovan, R. C., Petrache, A. M., et al. (2019). From extraction to advanced analytical methods: The challenges of melanin analysis. International Journal of Molecular Sciences, 20(16), 3943. https://doi.org/10.3390/ijms20163943

[16] Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal identification using molecular tools: A primer for the natural products research community. Journal of Natural Products, 80(3), 756–770. https://doi.org/10.1021/acs.jnatprod.6b01085

[17] Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., & Andersen, B. (2019). Food and Indoor Fungi (2nd ed.). Westerdijk Fungal Biodiversity Institute.

[18] Samson, R. A., Visagie, C. M., Houbraken, J., et al. (2024). Aspergillus, Penicillium and Talaromyces: Classification and Identification. Studies in Mycology

[19] Sambrook, J., & Russell, D. W. (2001). Molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor Laboratory Press.

[20] Schoch, C. L., Seifert, K. A., Huhndorf, S., et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proceedings of the National Academy of Sciences, 109(16), 6241–6246. https://doi.org/10.1073/pnas.1117018109

[21] Seelam, S. D., Agsar, D., Halmuthur, M. S., Shetty, P. R., Vemireddy, S., Reddy, K. M., Umesh, M. K., & Rajitha, C. H. (2021). Characterization and photoprotective potentiality of lime dwelling Pseudomonas-mediated melanin as sunscreen agent against UV-B radiations. Journal of Photochemistry and Photobiology B: Biology, 216, 112126.

[22] Singh, S., Nimse, S. B., Mathew, D. E., Dhimmar, A., Sahastrabudhe, H., Gajjar, A., Ghadge, V. A., Kumar, P., & Shinde, P. B. (2021). Microbial melanin: Recent advances in biosynthesis, extraction, characterization, and applications. Biotechnology Advances, 53, 107773. https://doi.org/10.1016/j.biotechadv.2021.107773

[23] Suwannarach, N., Kumla, J., Watanabe, B., Matsui, K., & Lumyong, S. (2019). Characterization of melanin and optimal conditions for pigment production by an endophytic fungus, Spissiomyces endophytica SDBR-CMU319. PLoS ONE, 14(9), e0222187.

[24] Tran-Ly, A. N., Reyes, C., Schwarze, F. W. M. R., & Ribera, J. (2020). Microbial production of melanin and its various applications. World Journal of Microbiology and Biotechnology, 36, 170. https://doi.org/10.1007/s11274-020-02941-3

[25] White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), PCR Protocols: A Guide to Methods and Applications (pp. 315–322). Academic Press.