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Optimization of Orange Pigment Yield Produced by *Monascus ruber* under Submerged Fermentation Conditions

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Abstract

This study was conducted to isolate and identify potent pigment producers. From thirty-three fungal isolates, one isolate (FPO2) which isolated from yoghurt samples was able to produce an extracellular orange pigment. The fungal isolate was identified as *M. ruber*. Among the tested culture media, potato dextrose broth (PDB) was the most suitable medium for the maximum orange pigment secretion. The supplementation PDB with mannitol at concentration 1.5% the maximum orange pigment was produced by *M. ruber*. On the other hand, the addition of any nitrogen source to the medium led to decrease in orange pigment production. Pigment production gradually increased as the incubation period increased, reaching the maximum value ($O.D_{460}$ 2.856) after 9 days. Additionally, 2% inoculum size was found to be optimum for pigment production under shaking conditions (150 rpm) at pH 7.0 when the fungus grown at 28 °C in darkness.

Key words: orange pigment, optimization, *Monascus* sp.

Introduction

Pigments are being used by mankind from primeval times for numerous purposes **(Bisht *et al.*, 2020)**. Knowledge of well-studied plant and animal sources must be expanded to include microorganisms, which have the potential to be a source for the bio-pigments production, in order to better understand the potential of colors derived from natural sources **(Sen *et al.*, 2019)**. A convenient alternative source of natural colors is microorganisms. Microbial pigments have many advantages over other natural pigments, including rapid growth, simple processing, and independence from seasonality and weather **(Aman Mohammadi *et al.*, 2022)**. Moreover, microbial pigments have many benefits such as supply sustainability; labor cost; yield; stability; cost efficiency as well as, ease of downstream treating **(Tuli *et al.*, 2015).**

Monascus is a genus of small, filamentous, saprophytic fungus that is members of the Eumycophyta, Ascomycotina, Plectomycetes, Eurotiales, and Monascaceae. *Monascus* pigments (MPs) are significant secondary metabolites of this species, an excellent potential pigment secration of certain *Monascus* species, for example, *Monascus ruber* and *Monascus purpureus* has been described **(Yang *et al.*, 2016).** Many secondary bioactive compounds, including pigment, are produced by *monascus* strains. MPs include six main structures, namely, double yellow pigments (ankaflavin and monascin), double orange pigments (monascorubin, rubropunctatin) and double red pigments (monascorubramine and rubropunctamine) **(Patakova 2013)**. According to **Zeng *et al.*, 2018**, substantial effort has been made to evaluate factors that effect of MPs production, such as pH, temperature, incubation period, dissolved oxygen, and nutritional requirements such as carbon and nitrogen sources to cultivate and to use this information to optimize the culture conditions on both submerged and solid state fermentation.

MPs have been used as a functional food additive for several thousands of years. Modern research found that MPs have many applications such as coloring agents in foodstuffs and texture industries, pharmacology, medicine and cosmetics **(Mostafa and Abbady 2014).** Moreover, MPs possessed a range of biological activities, such as antimutagenic and anticancer properties **(Hsu *et al.*, 2011)**, antimicrobial activities **(Vendruscolo *et al.*, 2015)** and antioxidant **(Pyo and Lee 2007)**.

The present work deals with an aim to isolate pigment-producing fungi and optimizing the

nutritional and environmental factors that required scale-up its maximum production.

Materials and methods

Isolation and purification of pigment-producing fungi

Eight food samples (pickles, biscuit, cake and yogurt) were collected from local markets at Al Qalyubia Governorate at September 2021. Isolation process was carried out using pouring plate method on potato dextrose agar (PDA) medium amended with 250 mg/L of chloramphenicol (to regulator bacterial growth). The plates were incubated at 28± 2 ºC for 7 days **(Nafady *et al*, 2014)**. The fungi presenting intense bright color in the medium were chosen and transferred to fresh rose bengal chloramphenicol agar plates then purified by single hyphal tip method **(Korhonen and Hintikka 1980)**. Pure cultures were kept on PDA slants for further studies.

Identification of pigment-producing fungi

To investigate the colonial morphology, the chosen pigment-producing fungal strain was cultivated on PDA plates for 7 days at 28 °C. The description included macroscopic features like color, size, form, pigment generation, and texture of the surface. A light microscope was used to examine the fungus' microscopic characteristics.

Inoculum preparation and measurements

About 100 mL of the PDB were transferred into 250 mL Erlenmeyer flasks, sterilized and inoculated with fungal mycelial plugs (6 mm in diameter) obtained from the margin of the growing colony on PDA at 28± 2 ºC for 7 days. The inoculated flasks were incubated with shaking at 150 rpm for 7 days at 28˚C. After incubation period, the contents of each flask were collected and centrifuged at 10,000 rpm for 10 minutes. Orange pigment production was indirectly evaluated by measuring the absorbance of the culture supernatant at 460 nm using a 6405 UV/Vis Spectrophotometer (Agilent Technologies, Santa Clara, California, USA) after the supernatant fluid was filtered through a filter paper (WhatmanNo.2). Dry biomass weight (DBW) was estimated by washing with deionized water and drying at 50°C for 48 h**.**

Optimization of pigment production

Effect of the type of culture liquid medium

Four productions liquid media PDB, Czapek dox broth (CDB), Sabouraud dextrose broth (SDB), and yeast extract peptone dextrose broth (YPDB) were tested to their suitability for orange pigment production by the selected fungi. All media were dispended in 250 mL flasks containing 100 mL medium. After sterilization, 1 mL (7×10^4 spores/ mL) from inoculum was taken into the medium and incubated in darkness under shaking (150 rpm) condition at 28°C for 10 days. Culture liquid medium that presented the highest yield of the pigment was chosen for further experiments.

Effect of carbon source

The suitable medium was separately supplemented with 2% concentrations of fructose, mannitol, sucrose, lactose, soluble starch, or glycerol instead of glucose (the medium's carbon source). The inoculated flasks were incubated at 28˚C for 10 days. The carbon source that presented the highest pigment production was selected for further experiments. The optimal carbon source was examined for the optimum concentration (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0%) under the same previous conditions.

Effect of nitrogen source

The medium with optimal carbon source was used to examine two groups of nitrogen sources (organic and inorganic compounds). Organic nitrogen sources included beef extract, peptone and yeast extract while inorganic nitrogen sources included ammonium nitrate, ammonium sulfate and sodium nitrate. The above-mentioned nitrogen sources were calculated to give equal final nitrogen concentration (1.2 gm. N/L)**.** After inoculation, flasks were incubated at 28˚C for 10 days.

Effect of different mineral salts

After determined the optimal source of nitrogen, five different mineral salts were individually supplemented to the medium. These minerals were ferrous sulphate, zinc sulphate, calcium sulfate, manganese sulphate, and copper sulphate. All were used at a concentration of 0.01 g/100 mL.

Effect of cultivation period

The modified medium that had been obtained from previous studies was inoculated with a fungal culture for various incubation periods, namely 5, 7, 9, 11, 13, and 15 days. The day with the highest pigment secretion was chosen.

Optimum inoculum size

To examination the effect of inoculum size on pigment production, fungal culture was inoculated in the modified medium with different inoculum sizes (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) and incubated for the cultivation period at 28˚ C.

Effect of agitation

For evaluation the agitation effect, fungal cultures were incubated in shaking incubator at 0, 50, 100, 150 and 200 rpm. The best agitation rate was selected for further studies

Effect of initial pH and incubation temperature

In this experiment, the selected liquid medium was adjusted from pH 4.0 to 9.0, inoculated with the fungal culture, incubated for the optimal incubation period and shaking rate. Similar approach was followed to check the optimal incubation temperature. The medium was incubated at 20, 24, 28, 32 and 36˚C under shaking. The pH and

temperature that presented the highest yield of the pigment was chosen for further experiments.

Effect of light

In order to study the effect of different wavelengths of light on fungus growth and pigment secretion, an experiment was carried out based on the principle that colored cellophane only transmits light of the corresponding color, excludes other colors in the spectrum. Fermentation flasks were wrapped in red, blue, orange and green colored cellophane; a light source (Philips CFL, 100 W) was placed near the flasks and kept in the incubator. The flasks were held directly under a light source to study the effect of keeping under direct lighting, and covered the flasks with aluminum foil to study the effect of keeping in complete darkness.

Results and discussions

Isolation and purification of pigment-producing fungi

In nature there are several microorganisms that can harvest pigments. These microorganisms can be found in food, plants, and soil, i.e. this work was focused on the isolation and purification of potent pigment-producing fungi. A total of thirty-three (33) fungal isolates were isolated on PDA due to their ability of pigment secretion. One fungal isolate coded as (FPO2) and obtained from yoghurt samples could produce an extracellular orange pigment as illustrated by Fig. (1)

Morphological characteristics

The fungal isolate was characterized and identified based on its morphological properties. In the macroscopic observation of the fungal isolate which exhibited orange color. Its colonies reached 2-3 cm diameter after 7days of incubation on PDA. It formed irregular spreading, Cottony, orange, reverse was orange with dark center and the media was pigmented with orange color as shown in **Fig (1)** B and C). In the microscopic observation of the fungal isolate, the asexual form with a chain of conidia and the sexual form with thin-walled ascocarps containing oval ascospores were observed. These characteristics, in addition to the culture characteristics, indicated that the obtained isolate was of *Monascus ruber***.**

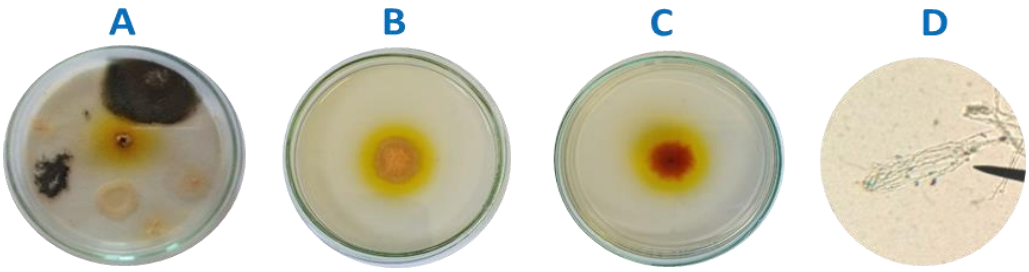

Fig. 1 Morphological characteristics of the orange pigments-producing fungal isolate. Colony growth was observed on PDA. (A) shows the ability of the fungal isolate to produce orange pigment,(B) show a front view of the growth and the plate cultures (C) show a reverse view of the growth after incubation for 7 days at 28 °C and (D) appearance under light microscope .

Effect of culture medium on pigment production

The effect of production media on orange pigment production by *M. ruber* was examined by four different media for their ability to support orange pigment production **(Fig. 2)**. Comparing among the tested four culture media, potato dextrose broth (PDB) was the most suitable medium for the highest orange pigment secretion ($O.D._{460}$ 1.982) by *M. ruber* under all tested incubation periods. On the other hand, Sabouraud dextrose broth (SDB) gave the lowest orange pigment secretion by *M. ruber* ($O.D._{460}$ 0.965). Additionally yeast extract peptone dextrose broth (YPDB) gave the highest yield of biomass (7.65 gm / 100 mL). Consequently, potato dextrose (PDB) broth was chosen for the succeeding experiments. The obtained results are in agreement with those described by **Neera *et al.*, 2017** found that PDB and a synthetic medium (composition gL^{-1}: glucose, 30; MSG, 1.5; KH_2PO_4, 2.5; $MgSO_4$.$7H_2O$, 0.5 and $FeSO_4$. $7H_2O$) gave high yields of pigment production. As well as, the growth medium of the fungus has great effect on growth of the fungal isolates and in production of secondary metabolites **(Gmoser *et al.*, 2017; Pisareva and Kujumdzieva 2010).**

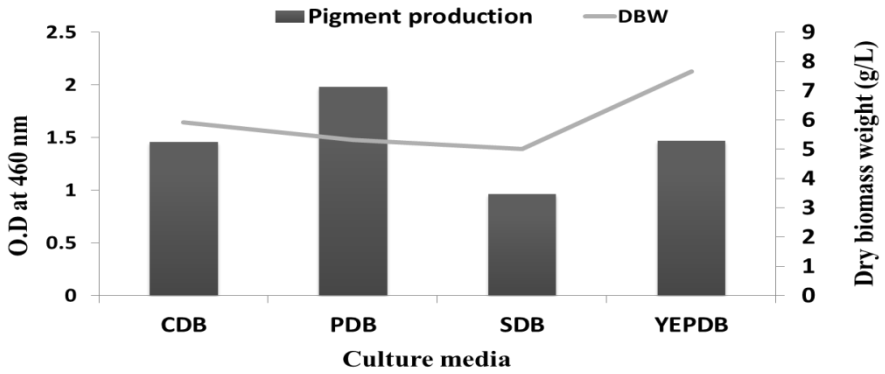

Fig. 2 Effect of culture liquid medium on orange pigment production by *M. ruber* submerged culture

Effect of carbon source

To determine the suitable carbon source for *M. ruber* to produce pigments, inoculum was cultured in PDB with several carbon sources e.i., glucose, fructose, mannitol, sucrose, lactose, soluble starch and glycerol. Sucrose gave the highest biomass weight (6.34 g/L) while the maximum pigment production ($O.D._{460}$ 2.714) was obtained from mannitol. On the other hand, starch was not suitable as carbon source for *M. ruber* to influence the orange pigment production. Results confirm that lactose and fructose are not efficient carbon source for fungi for the secretion of pigment. This result is in agree with that obtained by **Sankhyayan *et al.* (2019)** who found that Lactose and fructose were shown to significantly inhibit pigment synthesis.

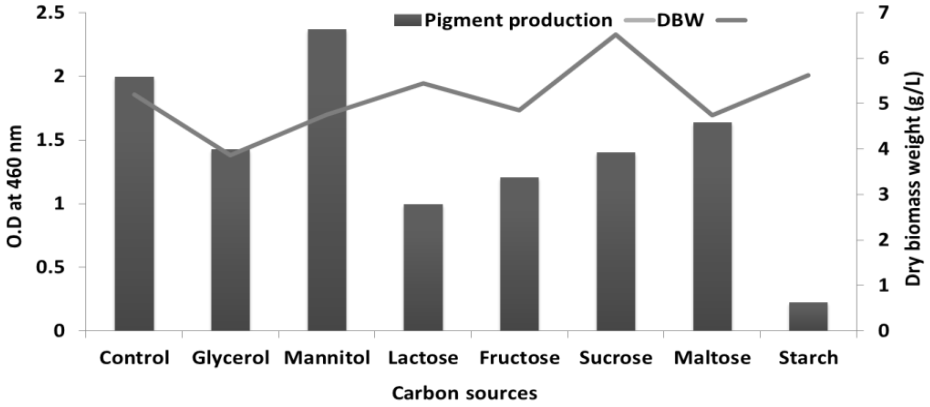

Fig. 3 Effect of carbon sources on orange pigment and biomass production by *M. ruber*

Effect of mannitol concentration

Since mannitol was found to be the most suitable carbon source for orange pigment production by *M. ruber*, it was of interest to investigate the effect of different mannitol concentrations on the production process. Mannitol concentrations from 0.5 to 4.0% were added to the optimized medium.

Results illustrated in **Fig. (4)** reveal that growth yield gradually increased with increasing in mannitol concentration. Moreover, concentration of 1.5% mannitol was found to be the most suitable for orange pigment production ($O.D_{460}$ 2.726) and by icreasing mannitol concentration over 1.5%, pigment production was gradually decreased. This result may be due to negative effect of high amounts of carbon and nitrogen sources on pigment formation, which likely due to the microorganism's catabolic inhibition under these conditions (**Santos-Ebinuma *et al.*, 2013)**. On the other hand, results reported by **Mousa *et al.* (2018)** who found that maltose is the ideal carbon source for pigment secration by *Monascus purpureus* at 1% concentration, and increasing maltose concentration to 5% stabilized productivity.

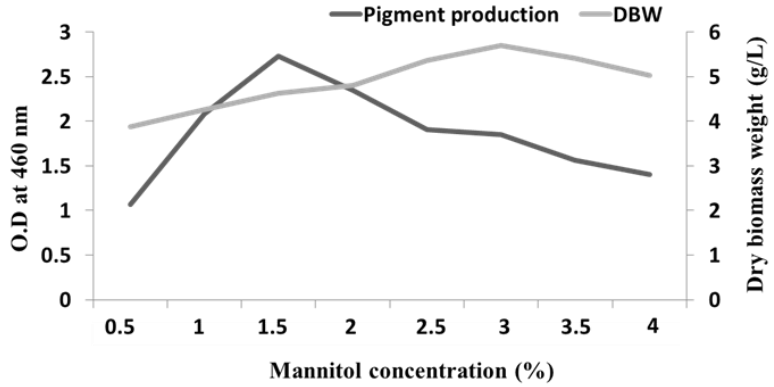

Fig. 4 Effect of mannitol concentration on orange pigment and biomass production by *M. ruber*

Effect of nitrogen sources

Results illustrated in **Fig. (5)** showed that addition of most examined nitrogen sources enhanced *M. ruber* growth. $NaNO_3$ gave the highest biomass production (6.67 g/L). On the other hand, data show that orange pigment production decreased by addition of nitrogen sources than control ($O.D._{460}$ 3.039). It is obvious from results that ammonium nitrate, peptone, ammonium sulphate and beef extract inhibited pigment production, while sodium nitrate and yeast extract decreased pigment production about 24.45 and 19.37 %, respectively than control (Without adding any nitrogen source).

Simallar results were obtained according to **Cho *et al.* (2002)** they reported that red pigment production by *Paecilomyces sinclairii* was severely suppressed by soy peptone or malt extract,. Also, **Gunasekaran and Poorniammal (2008)** discovered that utilizing soy peptone, beef extract, or potassium nitrate as a nitrogen source greatly suppressed red pigment formation by *Penicillium* sp.

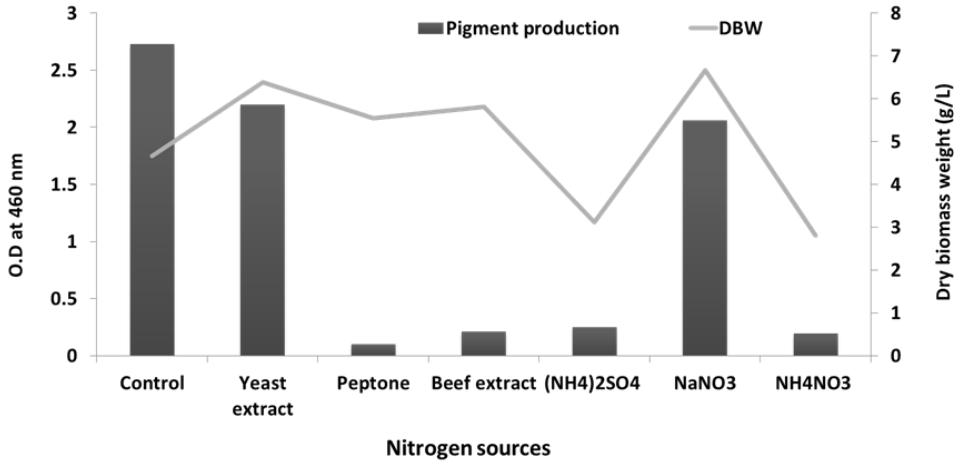

Fig. 5 Effect of nitrogen sources on orange pigment and biomass production by *M. ruber*

Effect of different mineral salts

Mineral salts are a significant component influencing color synthesis in a variety of microorganisms. Some of them, such as Mg^{2+} and Zn^{2+} ions, were important in increasing biomass and pigment synthesis. *M. ruber* was grown in a basal medium containing several metal salts to find the best metal salts for pigment synthesis. Five metal salts i.e., $CaSO_4$, $FeSO_4$, $MgSO_4$, $CuSO_4$, and $ZnSo_4$, have impact on biomass increase but reduce pigment synthesis. $MgSO_4$ produced the greatest biomass weight (6.41g/L) than any metal salt tested. On the other hand, data show that orange pigment production decreased by addition of all mineral salts sources than control (Without mineral salts**)**.

In the synthesis of pigments, minerals are essential. In liquid media, Zn (2×10^{-3} M and 3×10^{-3} M) inhibited the growth, however in solid media, strong development and coloration was observed **(Joshi 2013).**

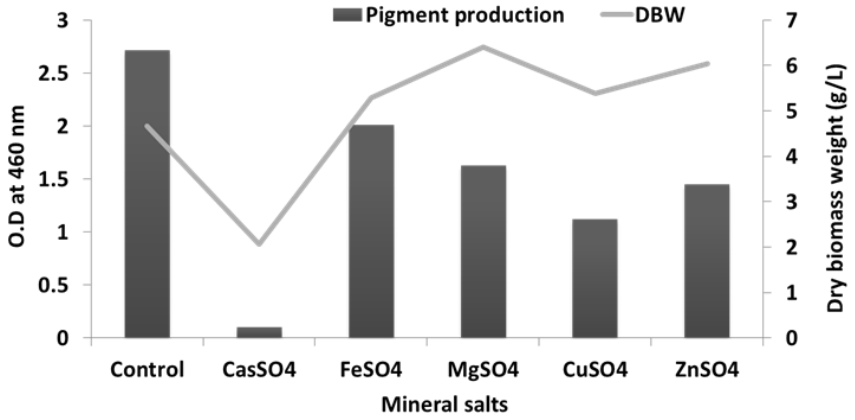

Fig. 6 Effect of different minerals on orange pigment and biomass production by *M. ruber*

Effect of cultivation period

It is crucial commercially for industrial fermentation to quickly harvest the end products. In order to find the best time for the product to be maximized, an experiment was conducted to know the rate of pigment creation throughout various fermentation periods. Data in **Fig. (7)** indicated that the rate of pigment synthesis grew progressively as incubation period increased, reaching its highest value ($O.D._{460}$ 2.856) after 9 days. These results In contradiction with the results obtained by **Babitha *et al*. (2006)** they found that *Monascus purpureus* produced its most pigment after six days of incubation. Additionally, **Silbir and Goksungur (2019)** noted that *Monascus purpureus* biomass peaked after 7 days of incubation and subsequently started to decline.

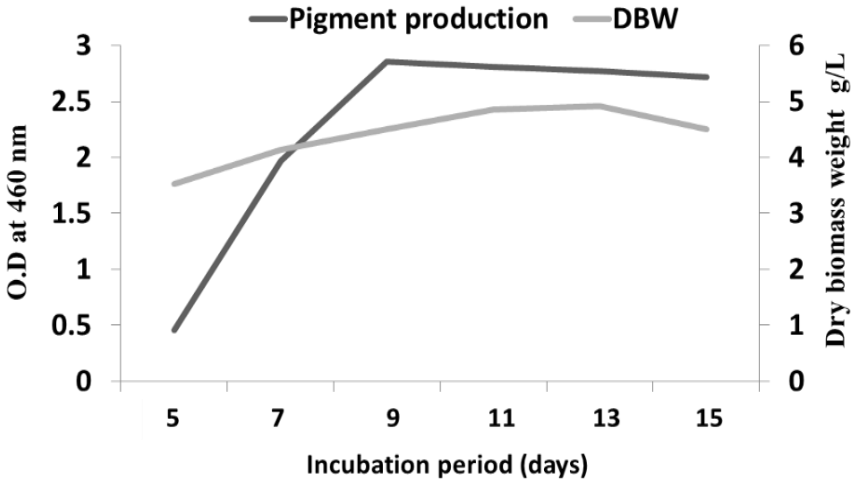

Fig. 7 Effect of cultivation period on pigment and biomass production by *M. ruber*

Inoculum size

The inoculum volume is one of the most important factors that affect the pigment yield. Inoculation was performed with inoculum volumes from 0.5 - 3%. The cultures were grown under the previously optimized conditions. It is seen from the obtained results which graphically illustrated in **Fig. (8)** the production of orange pigments increased by increasing the inoculum size up to 2% and decreased after that. Therefore, 2% inoculum was selected as the best for the tested fungus strain. The obtained results corresponded with **Babitha *et al*. (2007)** they showed that large inocula sizes may enhance biomass but limit pigment synthesis. The inhibition of pigment secration was caused by an absence in the culture medium of specific chemicals that were consumed by the high biomass.

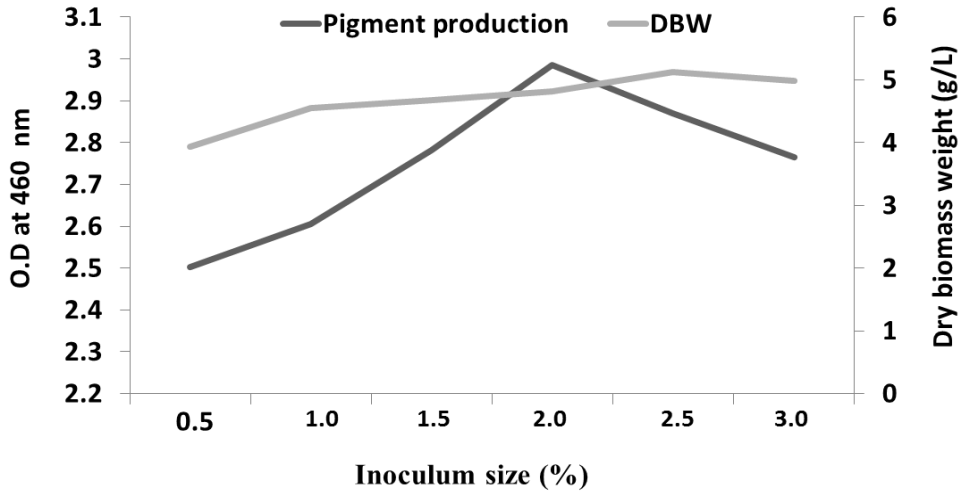

Fig. 8 Effect of inoculum size on pigment and biomass production by *M. ruber*

Effect of agitation rate

Results shown in **Fig. (9)** indicated that high biomass growth reached its highest value (5.97 g/L) at 100 rpm, whereas the rate of pigment production increase gradually as agitation increased, reaching its maximum value ($O.D._{460}$ 2.991) at 150 rpm. The findings are consistent with those published by **Méndez *et al.* (2011)** they reported that a high pigment production is not always related to a high biomass growth.

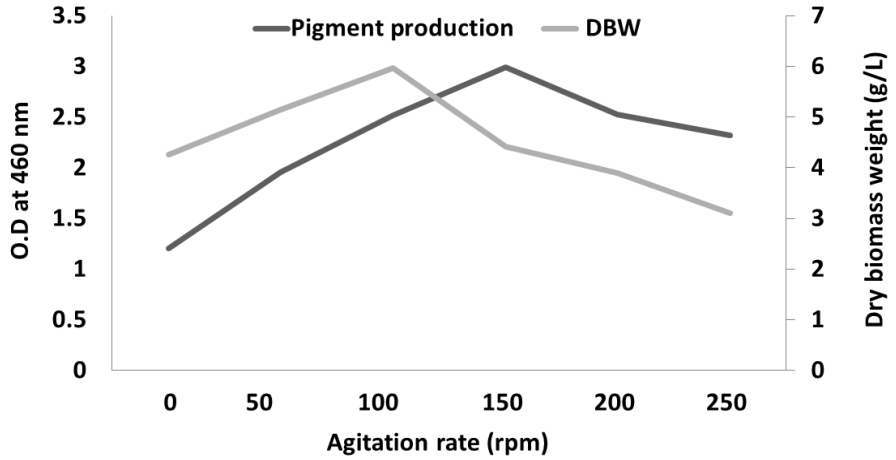

Fig. 9 Effect of agitation on orange pigment and biomass production by *M. ruber*

Initial pH

It is well known that the initial pH of the medium, in which the organism grown, has a great influence on pigments production. As seen in **Fig. (10)**, the highest production of pigment ($O.D._{460}$ 3.305) was observed at the initial pH value of 7. The change of the pH value above or below 7 resulted in decreasing the production of extracellular orange pigment. A low amount of pigment formation was observed at pH 8.5 and 9. In the culture medium with pH 9.0, pigment production was found to be the lowest ($O.D._{460}$ 0.962). The results show that despite the growth rate have not been much affected; the initial pH has a decent effect on pigment synthesis.

These findings agree with those published by **Merlin *et al.* (2013)** and **Sethi *et al.* (2016)** they reported that microbes can only grow within a specific pH range, and the pH value also influences how metabolites are formed. Additionally, the medium's initial pH can influence the activity of enzymes involved in the production of pigments, although the impact depends on the specific microbe used. **(Méndez *et al.*, 2011.** and **Afshari *et al.*, 2015)**

indicated that for *P. aculeatum*, 6.5 (nearly neutral pH) at 25 ^{o}C is the optimal pH for the formation of yellow pigments. As well As it, has been reported that during submerged culture, fungi produce a significant amount of pigment under acidic conditions, and that low pH inhibits conidia development and enhances pigment production, indicating that pH values affect the uptake of specific media constituents and enzyme activity involved in the biosynthesis of pigments **(Pandey *et al*., 2018).**

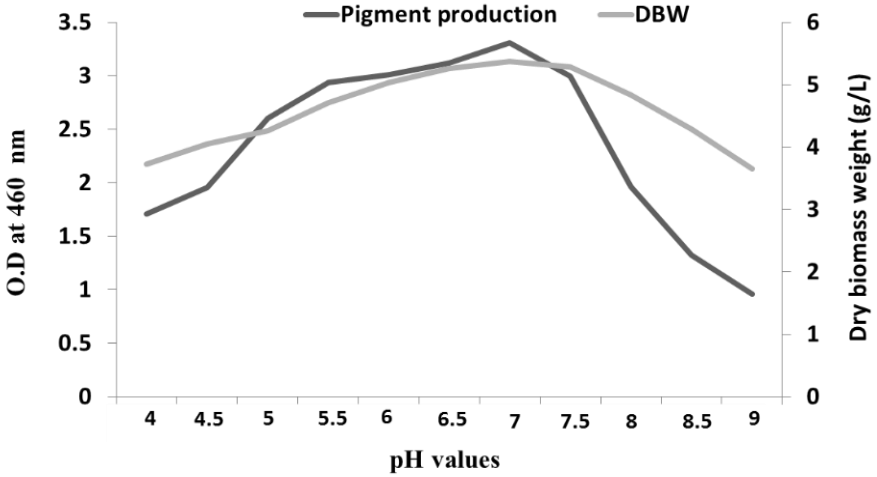

Fig. 10 Effect of pH on orange pigment and biomass production by *M. ruber*

Effect of incubation temperature

Temperature also affects fungal growth and pigment production as shown in **Fig. (11)**. the temperature which selected for present study ranges between 20-36 °C for optimization of pigments from fungal strain. The results show that production of orange pigment was increased gradually by increasing the incubation temperature from 20 to 28 ˚C and then gradually decreased with increasing the incubation temperature up to 28 ~ 36 ˚C. so 28 ˚C was considered as optimal temperature, which gave maximal orange pigment ($O.D._{460}$ 3.314) while, maximum biomass weight (5.58 gm/100 mL) was recorded at 32˚C as showed in **Fig. (11)**. According to **Zahan *et al.* (2020)**, *Penicillium minioluteum* ED24 should maintain an incubation temperature of approximately 30° C to produce pigment. Similar results were described by **Afshari *et al.* (2015)** they found that *Penicillium aculeatum* ATCC 10409 produced the most yellow pigment at 30 °C. **Bhosale (2004)** described that for low temperatures might reduce the rate at which nutrients are taken up from the environment, which would slow down metabolic activities such protein synthesis. In order to compensate the decline of biological pathway functionality caused by environmental changes, the pigments have therefore increased.

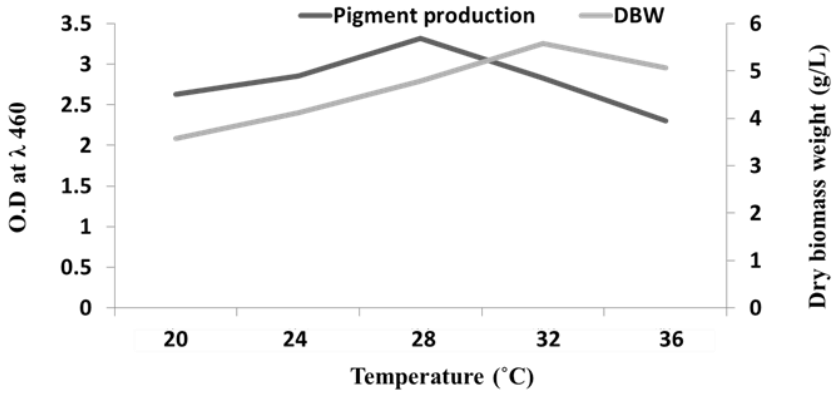

Fig. 11 Effect of temperature on orange pigment and biomass production by *M. ruber*

Effect of light

The level of pigment production decreased under the influence of blue light ($O.D._{460}$ 0.979) and red light ($O.D._{460}$ 0.718), the lowest yield was observed in green light ($O.D._{460}$ 0.488) and orange light ($O.D._{460}$ 0.354). A high level of pigment yield was observed in darkness ($O.D._{460}$ 3.325). Therefore, extracellular pigment changes rapidly depending on the light. The result indicates that pigment creation by M. ruber, which varies, is also connected with light exposure. According to **Blumenstein *et al.*, 2005** study the concentration of secondary metabolites (Gamma-Aminobutyric Acid (GABA) and red pigments) varies based on the color of the light and is particularly sensitive to sunshine.

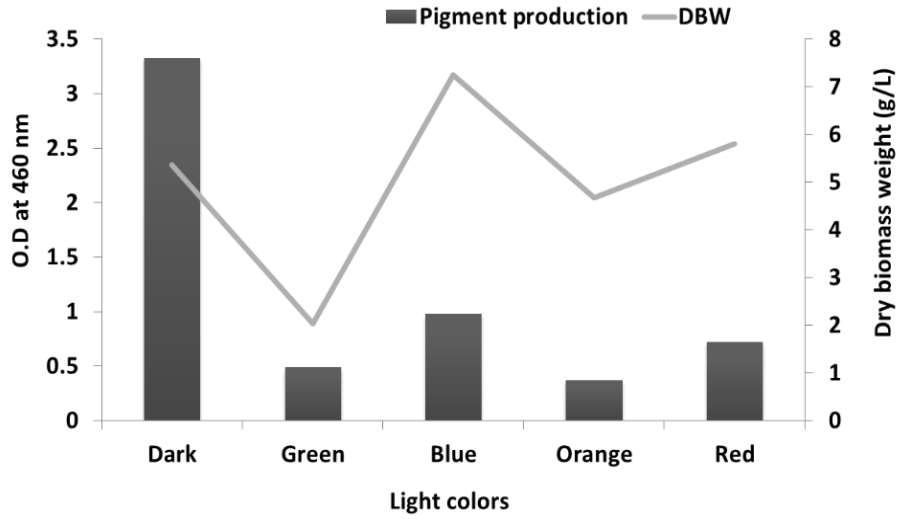

Fig. 12 Effect of light on orange pigment and biomass production by *M. ruber*

Conclusion

Production of orange pigment by *M. ruber* strain was controlled by many factors in the culture media. According to the types of carbon and nitrogen sources, mineral salts, and pH of the culture medium, production rates were increased or decreased. From obtained results it could be concluded that peptone, beef extract, $(NH_4)_2SO_4$, and NH_4NO_3 were not suitable nitrogen sources for the production of orange pigment while *M. ruber* was able to use a variety of sugars as carbon sources. All metal ions reduce the level of orange pigment production. The pH range of neutral was preferred for mycelial growth and pigment production, where the fungus produced its highest production at pH 7. Finally, *M. ruber* produced the highest orange pigment when it was grown in a modified PDB (15 g/l mannitol and adjusted the initial medium pH to 7 at 28 °C for 9 days).

Reference

Afshari, M.; Shahidi, F., Mortazavi, S. A., Tabatabai, F. and Es'haghi, Z. (2015). Investigating the influence of pH, temperature and agitation speed on yellow pigment production by *Penicillium aculeatum* ATCC 10409. Natural product research, 29(14): 1300-1306.

Aman Mohammadi, M.; Ahangari, H.; Mousazadeh, S.; Hosseini, S.M. and Dufossé, L. (2022). Microbial pigments as an alternative to synthetic dyes and food additives: A brief review of recent studies. Bioprocess Biosyst. Eng. 45: 1–12.

Babitha, S.; Soccol, C. R. and Pandey, A. (2006). Jackfruit seed-A novel substrate for the production of *Monascus* pigments through solid-state fermentation. Food Technology and Biotechnology, 44 (4): 465–471

Babitha, S.; Soccol, C. R. and Pandey, A. (2007). Effect of stress on growth, pigment production and morphology of *Monascus* sp. in solid cultures. Journal of Basic Microbiology, 47(2): 118-126.

Bhosale, P. (2004). Environmental and cultural stimulants in the production of carotenoids from microorganisms. Applied Microbiology and Biotechnology, 63: 351-361.

Bisht, G.; Srivastava, S.; Kulshreshtha, R.; Sourirajan, A.; Baumler, D. J. and Dev, K. (2020). Applications of red pigments from psychrophilic *Rhodonellum psychrophilum* GL8 in health, food and antimicrobial finishes on textiles. Process Biochemistry 94:15-29

Blumenstein, A.; Vienken, K.; Tasler, R.; Purschwitz, J.; Veith, D.; Frankenberg-Dinkel, N. and Fischer, R. (2005). The *Aspergillus nidulans* phytochrome FphA

represses sexual development in red light. Current biology: CB, 15(20): 1833–1838.

Cho, Y. J.; Park, J. P.; Hwang, H. J.; Kim, S. W.; Choi, J. W. and Yun, J. W. (2002). Production of red pigment by submerged culture of *Paecilomyces sinclairii*. Letters in Applied Microbiology, 35(3): 195-202.

Gmoser, R.; Ferreira, J. A.; Lennartsson, P. R. and Taherzadeh, M. J. (2017). Filamentous ascomycetes fungi as a source of natural pigments. Fungal biology and biotechnology, 4: 1-25.

Gunasekaran, S. and Poorniammal, R. (2008). Optimization of fermentation conditions for red pigment production from *Penicillium* sp. under submerged cultivation. African journal of Biotechnology, 7(12): 1894-1898.

Hsu, Y. W.; Hsu, L. C.; Liang, Y. H.; Kuo, Y. H. and Pan, T. M. (2011). New bioactive orange pigments with yellow fluorescence from Monascus fermented dioscorea. J. Agric Food Chem. 59: 4512–4518

Joshi, V. K.; DeventraAttri,; AnjuBala, and ShashiBhusan. (2013). Microbial pigments, Indian journal of Biotechnology, 2: 362-369.

Korhonen, K. and Hintikka, V. (1980). Simple isolation and inoculation methods for fungal cultures. Karstenia, 20(1): 19-22.

Mendez, A.; Pérez, C.; Montañéz, J. C.; Martínez, G. and Aguilar, C. N. (2011). Red pigment production by *Penicillium purpurogenum* GH2 is influenced by pH and temperature. Journal of Zhejiang University Science B, 12(12): 961-968.

Merlin, J. N.; Christhudas, I. V. S. N.; Kumar, P. P. and Agastian, P. (2013). Optimization of growth and bioactive metabolite production: *Fusarium solani*. Asian J. Pharm. Clin. Res., 6(3): 98-103.

Mostafa, M. E. and Abbady, Ma. (2014). Secondary metabolites and Bioactivity of the Monascus pigments review article. Glob. J. Biotechnol. Biochem. 9 (1): 01–13.

Mousa, Shaimaa A.; Abdou, D.; Mohamed, G. A.; Abo-El- Seoud, M. A.; Karam Eldin, A. Z. A. and El-mehalawy, A. A. (2018). Production of red pigment by *Monascus purpureus* Nrrl 1992 under submerged and solid-state fermentation. Egyptian Journal of Microbiology, 53(1): 83-94.

Nafady, N. A.; Morsy, F. M.; Bagy, M. H.; Abd-Alla, M. H. and Moukabel, G. A. (2014). Fungal diversity in *Zea mays* L. plants and the ability of some isolated *Aspergillus terreus* isolates to produce riboflvin, Fungal Diversity, 43:1-25.

Neera, D. K.; Ramana, K. V. and Sharma, R. K. (2017). Optimization of *Monascus* pigment production and its antibacterial activity. International Journal of Current Research of Bioscience and Plant Biology, 4(3):71 -80.

Pandey, N.; Jain, R.; Pandey, A. and Tamta, S. (2018). Optimisation and characterisation of the orange pigment produced by a cold adapted strain of *Penicillium* sp. (GBPI_P155) isolated from mountain ecosystem. Mycology, 9(2): 81-92.

Patakova, P. (2013). Monascus secondary metabolites: production and biological activity, Journal of Industrial Microbiology and Biotechnology, 40 (2): 169-181.

Pisareva, E. I. and Kujumdzieva, A. V. (2010). Influence of carbon and nitrogen sources on growth and pigment production by *Monascus pilosus* C1 strain. Biotechnology & Biotechnological Equipment, 24(1): 501-506.

Pyo, Y. H. and Lee, T. (2007). The potential antioxidant capacity and angiotensin I-coverting enzyme inhibitory activity of *Monascus*-fermented soybean extract: evaluation of *Monacus*-fermented soybean extract as multifunctional food additives. J. Food Sci. 72: 218–223

Sankhyayan, M.; Walia, A. and Putatunda, C. (2019). Production of red pigment from fungal isolate DMMS-1. Int. J. Curr. Microbiol. App. Sci, 8(4): 2839-2846.

Santos-Ebinuma, V. C.; Roberto, I. C.; Simas Teixeira, M. F. and Pessoa Jr, A. (2013). Improving of red colorants production by a new *Penicillium purpurogenum* strain in submerged culture and the effect of different parameters in their stability. Biotechnology progress, 29(3): 778-785.

Sen. T.; Barrow, C. J. and Deshmukh, S. K. (2019). Microbial pigments in the food industry—challenges and the way forward. Front. Nutr. 6:7.

Sethi, B. K.; Parida, P.; Sahoo, S. L. and Behera, B. C. (2016). Extracellular production and characterization of red pigment from Penicillium purpurogenum BKS9. Algerian Journal of Natural Products, 4(3): 379-392.

Silbir, S.; and Goksungur, Y. (2019). Natural red pigment production by *Monascus purpureus* in submerged fermentation systems using a food industry waste: Brewer's spent grain. Foods, 8(5): 161.

Tuli H. S.; Chaudhary P.; Beniwal V. and Sharma A. K. (2015). Microbial pigments as natural color sources: current trends and future perspectives. *Int.* J. Food Sci. Tech. 52: 4669–4678.

Vendruscolo, F.; Bühler, R. M.; Carvalho, J. C.; Oliveira, D.; Moritz, D. E.; Schmidell, W. and Ninow, J. L. (2015). Monascus: a reality on the production and application of microbial pigments. Appl. Biochem. Biotechno. 178; 211-223.

Yang, C.; Wu, X.; Chen, B.; Deng, S. S.; Chen, Z.E.; Huang, Y.Y. and Jin, S.S. (2016). Comparative analysis of genetic polymorphisms among *Monascus* strains by ISSR and RAPD markers, Journal of the Science of Food and Agriculture, 97 (2): 636-640.

Zahan, K. A.; Ismail, N. S.; Leong, C. R.; Ab Rashid, S. and Tong, W. Y. (2020). Monascorubin production by *Penicillium minioluteum* ED24 in a solid-state fermentation using sesame seed cake as substrate. Materials Today: Proceedings, 31: 127–135.

تحسين إنتاجية الصبغة البرتقالية التي ينتجها *Monascus ruber* تحت ظروف التخمير المغمورة

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أجريت هذه الدراسة لعزل وتحديد افضل الفطريات المنتجة للصبغات من 33 عزلة فطرية ، حيث وجد ان عزلة واحدة (FPO2) والتى تم عزلها من عينة زبادي لها القدرة على إنتاج صبغة برتقالية خارجية. تم تعريف العزلة الفطرية على أنها *M. ruber*. بالاضافة الى إختبار أربع بيئات غذائية مختلفة وكانت مرق دكستروز البطاطس (PDB) هى الافضل لإفراز الصبغة. كشفت دراسات التحسين أيضًا أن الحد الأقصى لإنتاج الصبغة البرتقالية بواسطة *M. ruber* يمكن تحقيقه في بيئة PDB مع اضافة سكر المانيتول بتركيز 1.5٪. من ناحية أخرى ، أدت إضافة أي مصدر نيتروجين إلى الوسط إلى انخفاض إنتاج الصبغة البرتقالية. كما زاد إنتاج الصبغة تدريجياً مع زيادة فترة الحضانة لتصل إلى أقصى قيمة (O.D$_{460}$ 2.856) بعد 9 أيام. بالإضافة إلى ذلك ، وجد أن حجم اللقاح 2٪ هو الأمثل لإنتاج الصباغ تحت ظروف الاهتزاز (150 دورة في الدقيقة). عند درجة حموضة 7.0 وعلى درجة حرارة 28 درجة مئوية وذلك في الظلام..