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PRODUCTION AND CHARACTERIZATION OF MICROBIAL DYE PRODUCED BY THE SAPROPHYTIC FUNGUS *Epicoccum* SP.

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ABSTRACT

This investigation is an attempt to study the production and characterization of dye produced by saprophytic fungi. From macerated plant materials (grains and straws of wheat and maize), twenty two fungal isolates were obtained. The most efficient isolate in producing pigment (designated as W5) was isolated from wheat grains. This isolate was identified as *Epicoccum* sp. The fungal pigment was produced by growing the isolate on the potato dextrose liquid medium. The produced dye was extracted using ethyl acetate. For optical characterization, color hue, UV/VIS spectra and the refractive index were determined. The IR spectrum was also conducted for primary chemical characterization. The electrical properties were also determined for the obtained material.

Keywords: Biomaterials, *Epicoccum* sp., pigment, optical properties, electrical properties, and IR spectrum

INTRODUCTION

Nowadays, microorganisms play a great role in biotechnology of cheaper and environmental friendly natural products (Adrio & Demain, 2003). The microbial pigments (bio dyes) has been obtained since long time ago and their interest has increased as alternative to potentially harmful and toxic synthetic ones (Cauizares-Villanueva *et al*.,1998). Ascomycetous filamentous fungi belonging to the genera *Penicillium*, *Epicoccum*, and *Monascus* provide a ready available source of natural colorants that should easily be produced in high yields (Mapari *et al*., 2005 and 2006).

The present work is the first step of a systematic study on production, characterization and applications of dye material(s) exogenously produced by the saprophytic fungus *Epicoccum* sp.

MATERIAL AND METHODS

1- Isolation and identification of dye-producing isolates:

On some disused plant waste materials collected in bare heaps, red-orange colored zones colonized by saprophytic fungal organisms were noticed. Pigmented portions of heaped wheat and maize grains and straws were used to isolate the inhabitant fungi. Under aseptic conditions, the macerated plant materials were spread onto the surface of the potato dextrose agar (PDA) medium in Petri dishes. The seeded dishes were incubated at 28^{o}C until pigmented colonies arose. The dye producing colonies were sub-cultured and purified using the hyphal tip technique (Dhingra & Sinclair 1995). The purified isolates were designated with code numbers and maintained on PDA slant medium until needed.

The growth morphology and the microscopic features of mycelium and spores were examined for identification according to Domsch *et al.*(1980) and Barnett & Hunter (1972).

2- Dye production and Extraction:

The isolate No W5 producing the most dens extra cellular dye was chosen for dye production. Ten of conical flasks of 500 ml capacity containing 100 ml of PD broth each were inoculated with 6 mm diameter disc cut from young culture growing on PDA medium. The inoculated flasks were statically incubated at room ambient conditions of 30±2 °C and 12.5 h summer daylight for 10 days. Cultures were collected, cheese-clothes filtrated and centerfugated at 6000 rpm for 10 min. Portions from the separated clear supernatant was tested against different organic solvents to extract the included dye material. Equal volumes of culture filtrate and the efficient solvent, ethyl acetate, were mixed in 500 ml separating funnel and thoroughly agitated. Funnel was left vertically until complete layer-separation took place. Extraction process was repeated using three ethyl acetate portions. The colored ethyl acetate fraction was evaporated in rotary evaporator at 40°C under vacuum until dryness. The obtained colored pigment was quantitatively re-dissolved in 70% ethyl alcohol. The resulted solution was used for the optical activity and electrical conductivity studies.

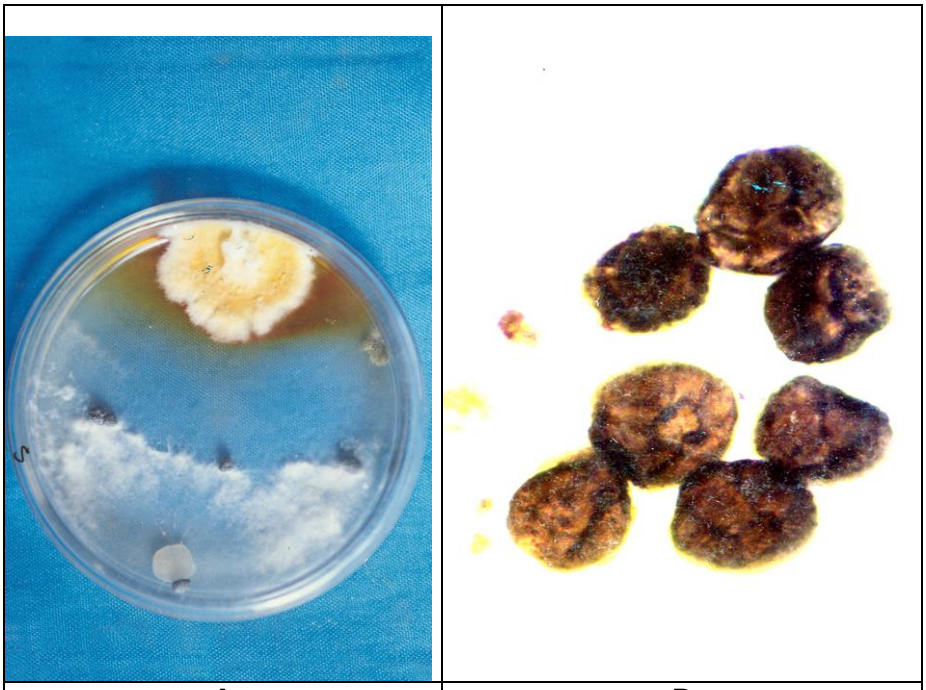
3- Optical and electrical measurements:

The color hue of the ethyl acetate extracted fungal dye was measured using Lovibond® comparator (Tintometer, Model F) in cell 5¼ inch as recommended by Lovibond (1915). The UV and VIS spectra were recorded using Jenway® 6105 UV/VIS Spectrophotometer while the IR spectrum was conducted on Perkin-Elmer 1430 IR spectrometer. The refractive index of the solution was determined using Abbe's digital refractometer (type Reichert ABBE Mark II). The DC conductivity of the solution was determined using the two probe technique. The absorbing edge, the linear absorption coefficient, the energy band gap and the electronic polarizability was estimated according to Vigil *et al*.(2000) and Kittel (1986).

RESULTS AND DISCUSSION

1- Isolation and identification of dye-producing isolates:

Isolation of the pigmented fungal isolates resulted in 22 fungal isolates which were designated with code numbers (M1 to M13 were isolated from maize and W1 to W9 were isolated from wheat). The most extra-cellular red orange dye production was observed by the fungal isolate No. W5, which was identified as *Epicoccum* sp. (Fig. 1) according to Domsch *et al.*(1980) and Barnett & Hunter (1972).



A **B**

Figure (1): Morphological feature of mycelial growth (A) and 1400 X magnified spores (B) of the isolate No W5 identified as *Epicoccum* sp.

2- Dye production and extraction:

After ten days growing period on PD broth medium, 85 mg/L of yellow reddish pigment material could be extracted using ethyl acetate. Chloroform, acetone or petroleum ether failed to extract any colored fractions. The obtained pigment completely dissolved in ethyl alcohol. Concentration of 100 ppm in 70% ethyl alcohol was used to determined the following optical and electrical characteristics

3- Optical and electrical measurements:

a) The color hue:

On the standard colored hue scale Lovibond with specific conditions as 35 Yellow shade and 5¼ inch inner cell, the suitable comparative range of color hue had 5 red (R) and 2 blue (B). This color shades could be added to the color palette of the natural colorants currently in use (Mapari *et al.,* 2006). Mapari *et al.* (2006) extracted a yellow pigment from *E. nigrum*, which was comparative in color to the yellowness of annatto.

b) UV/VIS spectra and refractive index:

Data illustrated in Figure (2) show the recorded UV and VIS transmission values for the obtained yellow colored pigment at the wavelength-spectra ranged from 200 nm to 900 nm. In the UV-spectrum range (200 – 400 nm), it is shown that the transmittance (T) is zero. This

behavior indicated that the UV radiation is totally absorbed in this pigment material. The transmittance begin to increase sharply from zero at ~ 460 nm until it approached maximum (91%) at ~ 580 nm, *i.e.* it is almost transparent at the wavelength range of 580-900 nm. The estimated absorbing edge for the tested sample at ~ 480 nm (2.58 eV) can be attributed to an electronic transition in this material. The linear absorption coefficient □ can be deduced from the relation:

$T = e^{-\square d}$ (1)

where d is thickness of the sample. Figure (3) shows the variation of the linear absorption coefficient (□□ versus wavelength for the yellow colored pigment. The energy band gap E_g was calculated according to Vigil *et al*.(2000) using the relation:

$$\alpha E = A(E - E_g)^2 \qquad (2)$$

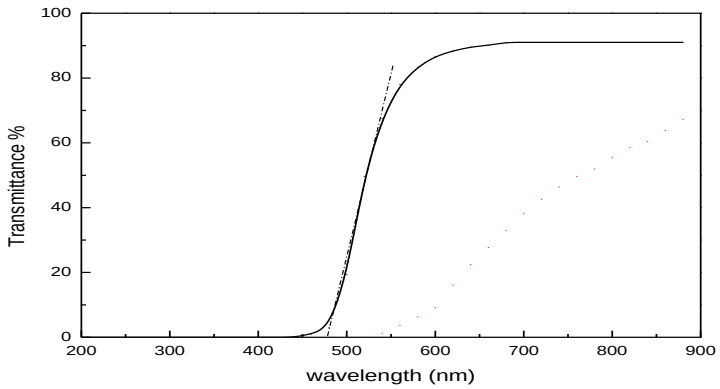


Figure (2): The UV-VIS spectra for the obtained yellow colored pigment.

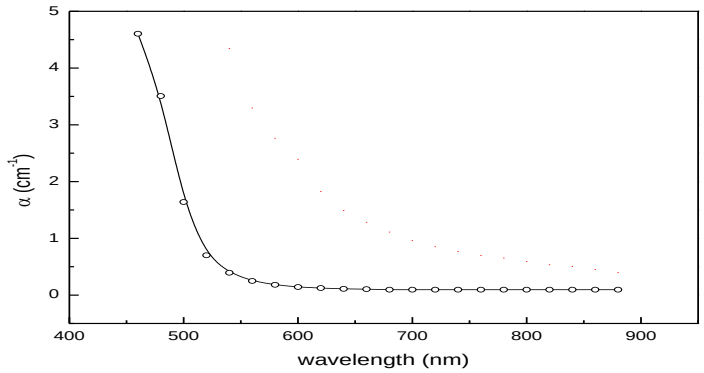


Figure (3): The linear absorption coefficient (α versus wavelength for the obtained yellow colored pigment.

where α is the linear absorption coefficient, $E = h\alpha$ is the photon energy. The energy band gap was determined by plotting $(\alpha h\alpha)^{1/2}$ as *y*-axis versus $h\alpha$ as *x*-axis and extrapolating the linear portion of the curve to intersect *x*-axis at E_g. Figure (4) shows the spectral energy dependence of $(\alpha h\alpha)^{0.5}$ for both samples. The energy band gap of the obtained yellow colored pigment is 2.2 electro-volt (eV) .

The electronic polarizability, α_m, per molecular weight, m, of the solution was calculated as mentioned by Kittel (1986) using Clausius-Mossotti relation:

$$\frac{n^2-1}{n^2+2} = \frac{4\pi}{3}\frac{\alpha_m}{m}N_A\rho \qquad (3)$$

where *n* is the refractive index of the liquid, N_A is Avogadro's number (6.02×10^{23} particles/mole), α is the density of the liquid. The refractive index was determined and found to be 1.358 for the obtained yellow colored pigment. The ratio α_m/m is found to be 1.332×10^{-25} cm^3 for the pigment. The electronic polarizability for the yellow colored pigment could be calculated which equal to 1.814×10^{-23} cm^3.

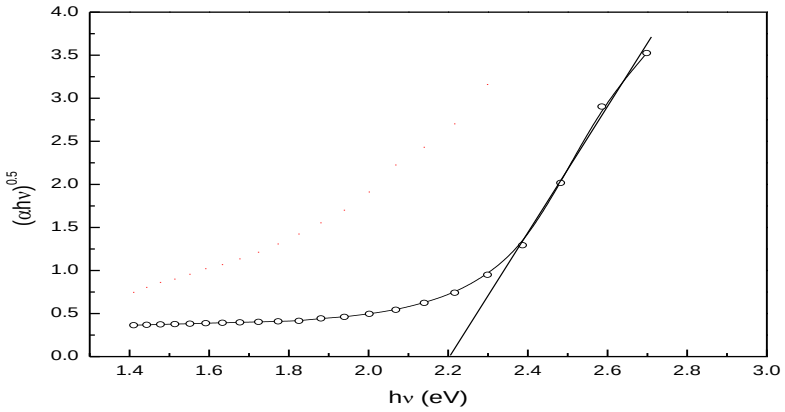


Figure (4): Spectral energy dependence of $(\alpha h\alpha)^{0.5}$ for the obtained yellow colored pigment.

c) IR spectra:

The IR spectrum was recorded for the yellow colored pigment as shown in Figure (5). The positions of the absorption bands and their intensities were recorded and listed in Table (1). The band 3424 cm^{-1}S is attributed to C─O in ether. The band 2989 cm^{-1}M is attributed to aromatic compound. The band 2151 cm^{-1} W is attributed to terminal alkynes, C≡C. The band 1640 cm^{-1} M is attributed to acyclic C─C. The band 1244 cm^{-1} W is attributed to aromatic ethers C─O. The band 1083 cm^{-1}W is attributed to fluoroalkanes C─F.

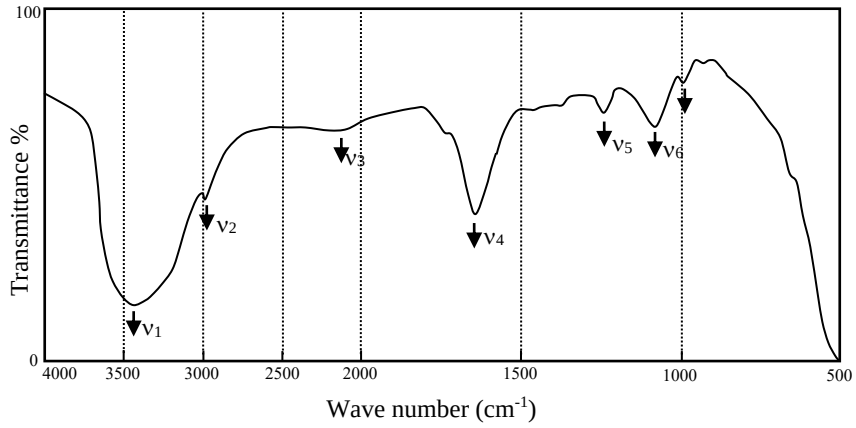
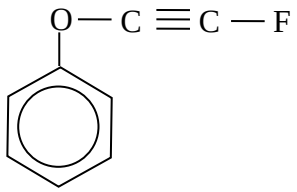


Figure (5): The IR spectrum for the obtained yellow colored pigment.

Table (1). The IR absorption bands for the yellow colored pigment and their intensities.

Wave number (cm-1)	**3424**	**2989**	**2151**	**1640**	**1244**	**1083**	**995**
I%	16.1	45.6	65.2	57.9	70.2	66.4	79.2

The suggested compound under investigation is C_8H_5OF with molecular weight 136.125 and structural formula:



d) DC electrical conductivity:

Figure (6) shows the variation of the DC electric current versus voltage across the obtained yellow colored pigment. The results show that the DC electric current increases linearly with increasing voltage. This indicates that the pigment obeys Ohm's law. The DC electric conductivity was determined and found to be 3.6×10^{-7} $\alpha^{-1}.cm^{-1}$ for the obtained yellow colored pigment. The low value of the DC electrical conductivity can be attributed to the value of the energy band gap.

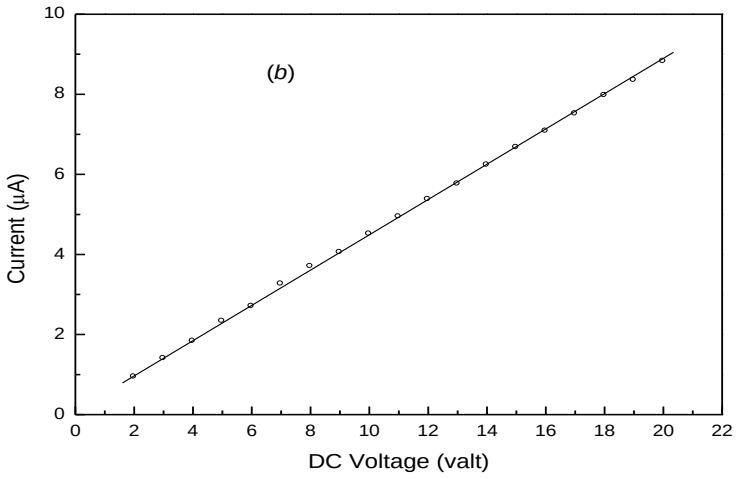


Figure (6): The DC electrical conductivity versus the voltage for the obtained yellow pigment.

Finally, it could be concluded that the obtained bio-derived yellow colored pigment can be considered as a perfect UV-absorber. It is expected to be a promising material in the field of UV-shielding industry. Studies on using and improving the quality of the pigment as a UV-shield film are intended.

REFERENCES

Adrio, J. L. and Demain, A. L. (2003). Fungal Biotechnology. Int. Microbial., 6 (3): 191-199.

Barnett, H. L. and Hunter, B. B. (1972) Illustrated genera of imperfect Fungi, 3rd edn., Burgess Publishing Company.

Cauizares-Villanueva, R. O.; Rios-Leal, E.; Olvera Ramirez, R.; Ponce Noyola, T. and Marquez Rocha, F. (1998). Microbial Sources of Pigments. Rev. Latinoam Microbiol. 4 (1-2): 87-107.

Dhingra, O. D. and Sinclair, J. B. (1995). Basic plant Pathology methods. Second Edition, CRC Press, Inc. Chapter 6: 217-266.

Domsch, K.H., Gams, W. and Anderson, T. H. (1980). Compendium of Soil Fungi. Academic press, London, N Y., toranto, Sydney, San Francisco.

Kittel, C. (1986). Introduction to Solid State Physics, Wiley, New York, Chapter 13.

Lovibond, J. W. (1915). Light and colour theories and their relation to light and colour standardization. University of California Libraries (Released online).

Mapari, S. A.; Nielsen, K. F.; Larsen , T. O.; Frisvad, J. C.; Meyer, A. S. and Thrane, U. (2005). Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. Curr. Opin. Biotechnol., 16 (2) 231-238.

Mapari, S. A.; Meyer, A. S. and Thrane, U. (2006) Colorimetric characterization for comparative analysis of fungal pigments and natural food colorants. J. Agric. Food Chem. 54 (9) 7027-7035.

Vigil, E.; Saadoun, L.; Ayllón, J. A.; Domònech, X.; Zumeta, I. and Clemente, R. R. (2000). TiO_2 thin film deposition from solution using microwave heating Thin Solid Films (365) 12-18.

إنتاج وتوصيف صبغة ميكروبية منتجة بالفطر الرمي *Epicoccum* sp.

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هذا البحث يمثل الخطوة الأولى لمجموعة دراسات متسلسلة تتناول إنتاج وتوصيف صبغة ميكروبية برتقالية حمراء تنتجها إحدى عزلات الفطر ***Epicoccum* sp** تمهيداً للاستخدامات التطبيقية لهذه الصبغة في المجالات التكنولوجية التي تستخدم فيها مثل هذه المواد المنتجة حيوياً.

ولقد تم تنمية هذه العزلة الفطرية على بيئة البطاطس والدكستروز السائلة, وتم استخلاص الصبغة من راشح المزرعة باستخدام خلات الإيثيل. ولتوصيف الخصائص الضوئية للمادة المستخلصة فقد تم تحديد اللون الطيفي لها، كذلك تم تقدير قيم امتصاصها للأشعة فوق البنفسجية والأشعة المرئية عند أطوال موجية مختلفة، وأيضاً تم تقدير معامل تشتيتها للضوء. وبتوقيع منحنى امتصاص هذه المادة للأشعة تحت الحمراء فقد ساعد ذلك على وضع تصور مبدئي للتركيب الكيماوي لهذه المادة.