



Research Article

MICROBIOLOGY

OPTIMIZATION AND CHARACTERIZATION OF ENDOPOLYSACCHARIDES FROM *PLEUROTUS Eryngii* AND ITS POSSIBLE APPLICATION

Eman H.F. Abd El-Zaher*, Alaa M. Abou Zied*, Heba M. Abdou**, Amira A. A. Mustafa*.

* Botany and Microbiology Department, Faculty of Science, Tanta University, Tanta, Egypt., ** Zoology Department, Faculty of Science, Alexandria University, Alexandria, Egypt

KEY WORDS

Endopolysaccharides,
production, structure,
biological activity,
Pleurotus eryngii,

ABSTRACT

The present study aimed to assess the optimization and characterization of endopolysaccharides from *Pleurotus eryngii*. The results of optimization of culture conditions showed that the highest growth and maximum production of endopolysaccharides of *Pleurotus eryngii* were achieved with using potato glucose medium, at 12 days incubation period, at temperature 30°C and with using inoculum size three discs (1cm in diameter for each) of *Pleurotus eryngii*. High performance liquid chromatography analysis detected that endopolysaccharides extracted from *Pleurotus eryngii* was composed of β -glucans, Chitin, Galactans and Mannans at concentration 36.20, 22.60, 21.60 and 19.60% respectively. Endopolysaccharides extracted from *Pleurotus eryngii* showed antioxidant activity.

Introduction

Mushroom, a protein-rich wonder food helps in addressing the issues of quality food, health, and environmental sustainability (Raut, 2019). Edible mushrooms have become attractive as “health foods” and as source materials for immunomodulators (Chen et al., 2018). They have beneficial effects such interventions ameliorate oxidative stress, hepatic lipid profiles, and reduce inflammation (Fontes et al., 2019). Mushroom cultivation is reported as

an economically viable bio-technology process for conversion of various lignocellulosic wastes. The mycelium growth of mushroom species is mainly dependents upon the substrate, nutrients and the growing conditions (Girmay et al., 2016). Several mushrooms belonging to *Pleurotus* species are confirmed to be producer of bioactive polysaccharide (Hao et al., 2017).

The main polysaccharides present in mushrooms are β -glucans with potential biological activities. β -glucans are soluble fibers with physiological functions, such as, interference with absorption of sugars and reduction of serum lipid levels (**Rahar et al., 2011**). Mushrooms act as antibacterial and immune system enhancer; additionally, they are important sources of bioactive compounds. As a result of these properties, some mushroom extracts are used to promote human health (**Valverde et al., 2018**).

Pleurotus genus is one of most extensively studied white-rot fungi due to its exceptional ligninolytic properties. It is an edible mushroom and it also has several biological effects, as it contains important bioactive molecules (**Bellettini et al., 2016**). The genus *Pleurotus* contain many biological compounds such as polysaccharides, enzymes, protein, dietary fibre and vitamins (**Shen et al., 2013**). It has been recently reported that many potential natural anti-oxidants are derived from lots of sources such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, herbs and mushrooms (**Soares et al., 2009; Lee and Yoon, 2009**).

The purpose of the present research was to evaluate the growth factors that might increase the productivity of endopolysaccharides. Also, characterization of endopolysaccharides by using HPLC. Finally, antioxidant activity were analysed in vitro.

Materials and methods

Producing organism and preservation

Pleurotus eryngii was obtained from agricultural research Centre (Central Laboratory for Agricultural Climate Giza, Egypt). *Pleurotus eryngii* was preserved on autoclaved slants containing potato glucose agar medium which consisted of (gm/L): 20 glucose, 200 potato and 20 agar. The slants were incubated at 30°C for 12 day and after incubation slants were stored in refrigerator at 4°C.

Determination of *Pleurotus eryngii* dry weight

Pleurotus eryngii was cultured in autoclaved 250 ml flasks each was contained 100 ml of potato glucose liquid medium and incubated at 25°C for 7 days. After incubation the fungal biomass was separated by filtration and washed several times with sterile distilled water and dried at 80°C until a constant weight (**Nour EL-Dein et al., 2004**).

Extraction of endopolysaccharides

Preparation of endopolysaccharide extract was carried out according to the method described by **Lung and Tasi (2009)**. Endopolysaccharide extract of *Pleurotus eryngii* was isolated from the fresh mycelia of *Pleurotus eryngii*. The fresh mycelia was washed with ethanol (95%) followed by distilled water, grinding with distilled water and was put in conical flask containing distilled water. The cultured mycelia was extracted with boiling water for 1 h and

was autoclaved at 121°C at 1.5 atm for 15 min and then filtered through filter paper (Whatman No. 1). Filtrates were precipitated with two volumes of 95% (v/v) ethanol and left overnight at 4°C. The resultant precipitate was recovered by centrifugation (Hettich EBA 12R, Germany) at 3000 rpm for 20 minutes (Wu et al., 2008).

Measurement of carbohydrate content

The content of endopolysaccharides was determined by phenol-sulfuric colorimetric method (Dubois et al., 1956) using glucose as standard.

Purification of endopolysaccharides

Crude endopolysaccharides were partially purified by dialysis membrane (Berg et al., 2007).

Effect of different physiological parameters on mycelial growth and endopolysaccharides production of *Pleurotus eryngii*

Different experiments were made to select the most favorable conditions for high production of endopolysaccharides dry weight.

Effect of different media on mycelial growth and endopolysaccharides production of *Pleurotus eryngii*

Three different liquid media; potato glucose, glucose yeast peptone and mushroom complete media were used. Inoculate each medium with one fungal disc (1cm in diameter) of *Pleurotus eryngii*. After incubation for 7 days at 25°C, the mycelial dry weight and endopolysaccharides were determined (Nour EL-Dein et al., 2004).

Effect of different incubation periods

In order to select the optimum incubation period for high production of endopolysaccharides from *Pleurotus eryngii*, potato glucose liquid medium flasks for different incubation periods (5, 10, 12 and 14 days) were carried out (Elshamy and Nehad, 2010). At the end of incubation periods the endopolysaccharides and growth biomass of *Pleurotus eryngii* were determined.

Effect of different temperature degrees

Different temperature degrees (20, 25, 30, 35, and 37°C) were tested for 12 day incubation (Elshamy and Nehad, 2010).

Effect of different inoculum size

Autoclaved potato glucose liquid medium flasks were inoculated separately with different inoculum number, one, two and three discs 1cm diameter size. The inoculated flasks were incubated at 30°C for 12 day.

Characterization of endopolysaccharides produced by *Pleurotus eryngii*

High Performance Liquid Chromatography Analysis

Endopolysaccharides were hydrolyzed following the method of Chen et al. (2005). Analysis of the carbohydrate in the filtrate was performed by using High Performance Liquid Chromatography (HPLC), Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID-10A Shimadzu detector.

Antioxidant assays

2,2-diphenyl-1-picrylhydrazyl(DPPH)

radical scavenging activity:

Total antioxidant activity of extracted endopolysaccharides was estimated according to the method described by **Ul-Haq et al. (2012)** using different endopolysaccharides concentrations (0.2, 0.4 and 0.8 mg) each dissolved in 1ml distilled H₂O.

RESULTS AND DISCUSSION

Effect of different media on mycelial growth and endopolysaccharides production of *Pleurotus eryngii*

Figure 1 showed that Potato Glucose liquid medium (PG) was the best medium for high mycelial biomass and endopolysaccharide extract of *Pleurotus eryngii* (PEPE) production which gave 29.72 ± 0.43 and 0.164 ± 0.01 mg/ml respectively. Mycelial biomass and PEPE concentration significantly ($P < 0.05$) increased on PG medium compared to Glucose Yeast Peptone Media (GYP) and Mushroom Complete Media (MCM). These results were agreed with **Hoa et al. (2015)** who found that potato dextrose medium was more suitable for the mycelium growth of *Pleurotus ostreatus*, this may be due to availability of required nutrients for mushroom *Pleurotus ostreatus* in potato medium.

Effect of different incubation periods

Incubation period has an essential role for high mycelia growth and high production of PEPE, So it was made to choose the optimum incubation period that gave high production of mycelial and PEPE dry weight. Figure 2

showed that mycelial biomass was 8.98 ± 0.006 mg/ml and PEPE dry weight was 0.348 ± 0.03 mg/ml after 12 day and then decreasing was recorded at 14 day. The optimum incubation time was 12 day and this was consistent with **Abd El-Zaher et al. (2015)** who showed that the GYP medium was used and incubated for 12 day to produce *Pleurotus ostreatus* and *Ganoderma resinaceum* polysaccharides.

Effect of different temperatures

It was evident that temperature at 30°C was the most suitable degree for the highest mycelial growth and maximum production of PEPE (Figure3). This was consistent with finding of **Elshamy and Nehad. (2010)** showed that the optimum temperature for polysaccharides formation by *Alternaria alternata* was found to be 30°C. Temperature 30°C was the optimum for polysaccharides production and mycelial growth from *P. commune* (**Abou Zied et al., 2017**).

Effect of different inoculum size

It was obvious that three discs sized 1cm in diameter from *Pleurotus eryngii* mycelia gave high mycelial and PEPE dry weight. Cultivation of three fungal discs with diameter 1cm from the tested organism on PG media gave high quantity of dry mycelia weight which was 19.13 ± 0.17 mg/ml and PEPE weight was 0.54 ± 0.04 mg/ml (Figure4). **Osman et al. (2014)** observed that increasing the inoculum size led to a significant increase in growth and polysaccharides production.

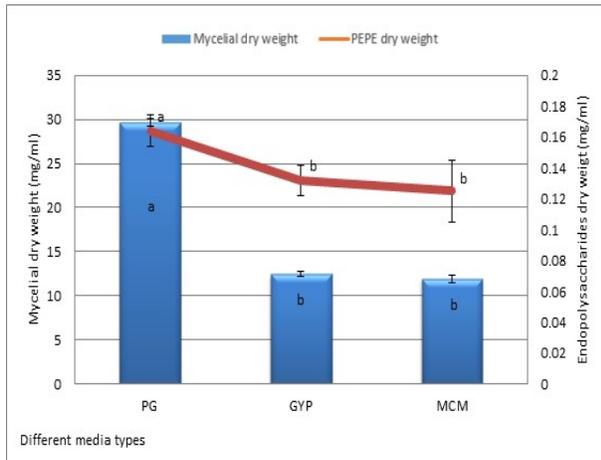


Fig. (1): Effect of different media on mycelial growth and endopolysaccharides production of *Pleurotus eryngii*.

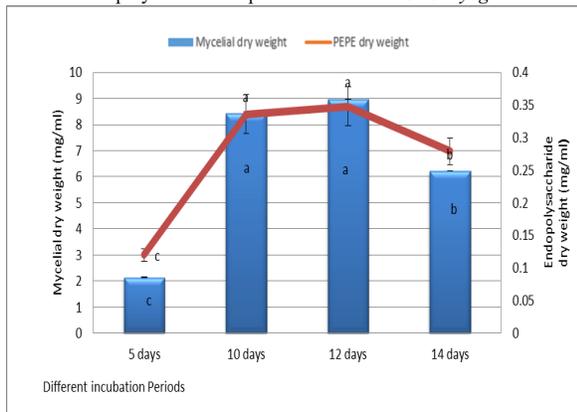


Fig. (2): Effect of different incubation periods on mycelial dry weight and endopolysaccharides production of *Pleurotus eryngii*.

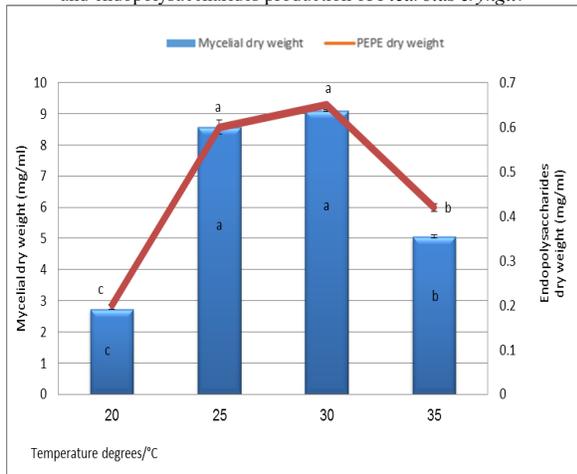


Fig. (3): Effect of different temperature degrees on mycelial and endopolysaccharides production dry weight of *Pleurotus eryngii*.

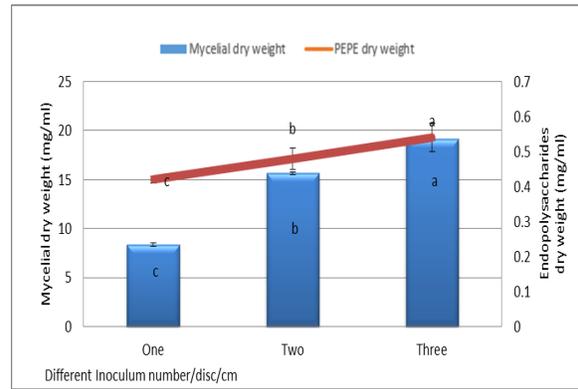


Fig. (4): Effect of different inoculum numbers on mycelial and endopolysaccharides production dry weight of *Pleurotus eryngii*.

Characterization of endopolysaccharides produced by *Pleurotus eryngii*

High Performance Liquid Chromatography Analysis

Endopolysaccharides were subjected to High Performance Liquid Chromatography analysis (HPLC). The HPLC chromatograph (Figure 5) showed that they consist of four peaks that referred to presence of β -glucans, Chitin, Galactans and Mannans For 1mg of endopolysaccharides structure analysis, the concentrations of β -glucans, Chitin, Galactans and Mannans were 36.20%, 22.60%, 21.60% and 19.60%. According to **Jahan and Singh (2019)** mushrooms are a good source of insoluble fiber in the form of chitin (polymer of N-acetyl-glucosamine) and non-starch polysaccharides like β -glucans. Also, *cultivated mushrooms Agaricus bisporus, Pleurotus ostreatus and Lentinula edodes* fruit bodies contain chitin (**Vetter, 2007**).

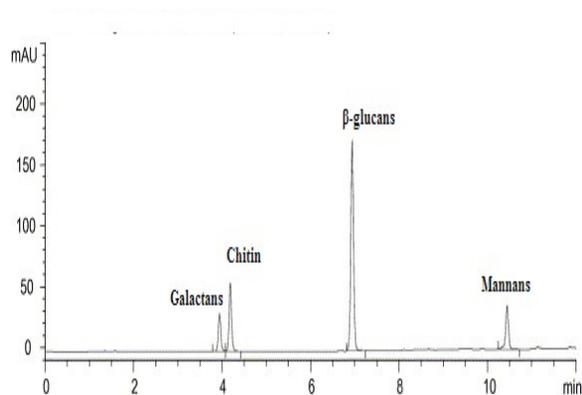


Fig. (5): HPLC of monomer of endopolysaccharides in culture filtrate produced by *pleurotus eryngii*.

Biological activities

DPPH scavenging activity of endopolysaccharides

DPPH method is usually to evaluate antioxidant activity of various natural compounds by reducing stable DPPH radicals to yellow-colored diphenylpicryl-hydrazine. DPPH radical scavenging ability is responsible for hydrogen donating efficiency of antioxidants. As shown in Figure (6) DPPH radical scavenging activity of endopolysaccharides increased gradually with increasing concentration. At 0.8 mg/ml scavenging effects of PEPE were 72.4%. It has been reported by **Alam et al. (2011)** that the higher scavenging activity of the acetone extract of *P. citrinopileatus* might be due to the hydrogen-donating components contained within it. The present results also agree with the results of **Li et al. (2012)** who suggested that all the polysaccharides from *Hypsizygus marmoreus*, *Lentinus edodes*, *Russula vinosa* Lindblad, *Hohenbuehelia serotina*, *Auricularia auricula* and *Hericium erinaceus* had

significant antioxidant capacities (ranged from 18.54% to 100%) at concentration of 20 mg/ml. In addition **Sharma et al. (2015)** stated that the intracellular polysaccharides of both strains *P. ostreatus* PBS281009 and M2191 were found to show a higher DPPH scavenging activity than those of the exopolysaccharides synthesized. Also, **Zhao et al. (2005)** reported that radical scavenging ability is responsible for hydrogen donating efficiency of antioxidants. Therefore, the antioxidant activity may be attributed to the proton donating ability, indicating that it may contain lots of reductones to react with radicals to stabilize and terminate radical chain reactions.

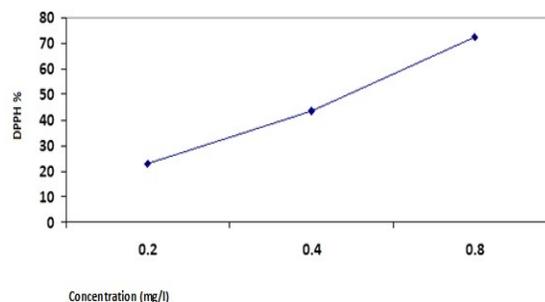


Fig. (6): Antioxidant activity of endopolysaccharides production from *Pleurotus eryngii*.

Conclusion and Recommendation

The current study indicates that endopolysaccharides extracted from *Pleurotus eryngii* has efficient scavenging activity. Finally, it can be recommended that supplementation of natural products as *Pleurotus eryngii* mushroom is an important powerful antioxidant and can be used for medical purposes. Future studies are needed to evaluate the feasibility of *Pleurotus eryngii*

extract for developing potent antioxidant drugs and detect their biological activities.

References:

- Abd El-Zaher, E.H.F.; Mostafa, H.A.A.; Shalaby, E. and Attia, W.Y.F. (2015). A comparative study on endopolysaccharides and exopolysaccharides production from some basidiomycetes with studying the antioxidant and anti-tumour effects of *pleurotus ostreatus* mushroom. Egypt. J. Exp. Biol., 11(1): 1-14.
- Abou Zied, A. M.; Abd El-Zaher, E. H.F. ; Ibrahim, H. A.H. and Hammad, T. A. (2017). Exopolysaccharides Production and Characterization from Marine-Derived *Penicillium commune* KP942881.1 with Some Medical Potential Applications. Egypt. J. Bot., 57:17 – 30.
- Alam, N.; Yoon, K.N.; Lee, K.R.; Kim, H.Y.; Shin, P.G.; Cheong, J.C.; Yoo, Y.B.; Shim, M.J.; Lee, M.W. and Lee, T.S. (2011). Assessment of antioxidant and phenolic compound concentrations as well as Xanthin oxidase and Tyrosinase inhibitory properties of different extracts of *Pleurotus citrinopileatus* fruiting bodies. Mycobiology, 39(1):12-19.
- Bellettini, M.B.; Fiorda, F.A.; Maieves, H.A.; Teixeira, G.L.; Avila, S.; Hornung, P.S.; Junior, A.M. and Ribani, R.H. (2016). Factors affecting mushroom *Pleurotus* spp. Saudi J. Biol. Sci., 26 (4) : 633-646.
- Berg, J. M. (2007). Biochemistry, 6th ed. New York: W.H. Freeman and Company. p. 69.
- Chen, S.C.; Lu, M.K.; Cheng, J.J. and Wang, D.L. (2005). Antiangiogenic activities of polysaccharides isolated from medicinal fungi. FEMS Microbiol. Lett., 249(2): 247–254.
- Chen, Q.; Qi, C.; Peng, G.; Liu, Y.; Zhang, X. and Meng, Z. (2018). Immune-enhancing effects of a polysaccharide PRG1-1 from *Russula griseocarnosa* on RAW264.7 macrophage cells via the MAPK and NF-κB signalling pathways. Food Agr. Immunol., 29(1): 833-844.
- Dubois, M.; Gilles, K. A.; Hamilton, J.K.; Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.
- ELshamy, A.R. and Nehad, E.A. (2010). Optimization of polysaccharide production by *Alterneria alternate*. gate2 biotech. com., 1(9): 1-6.
- Fontes, A.; Pages, M.A.; Oliveira, P.J.; Santos, J.R.; Zischka, H. and Azul, A.M. (2019). Antioxidant Versus Pro-Apoptotic Effects of Mushroom-Enriched Diets on Mitochondria in Liver Disease. Int. J. Mol. Sci., 20: 1-35.
- Girmay, z.; Gorems, W.; Birhanu, G. and Zewdie, s. (2016). Growth and yield performance of *Pleurotus ostreatus* (*Jacq. Fr.*) Kumm (*oyster mushroom*) on different substrates. AMB Expr, 6(87): 1-7.
- Hao, C.; Yang, J.; Liang, T.; Zhang, J. and Sun, R. (2017). Structural elucidation and morphological observation of a polysaccharide from *Pleurotus eryngii* obtained by alkaline extraction. J. Carbohydr. Chem., 36 (3): 129-140.
- Ho, H.T.; Wang, C.L. and Wang, C.H. (2015). The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology, 43(4): 423-434.
- Jahan, A. and Singh, B.K. (2019). Mushroom Value Chain and Role of Value Addition. I.J.B.R., 9(1): 5-14.
- Lee, K.H. and Yoon, W.H. (2009). Production of exopolysaccharide from submerged culture of *Grifola frondosa* and its antioxidant activity. Food Sci. Biotechnol., 18(5): 1253-1257.
- Li, N.; Li, L.; Fang, J.C.; Wong, J.H.; Ng, T.B.; Jiang, Y. ; Wang, C.R. ; Zhang, N.Y. ; Wen, T.Y. ; Qu, L.Y. ; Lv, P.Y. ; Zhao, R. ; Shi, B. ; Wang, Y.P. ; Wang, X.Y. and Liu, F. (2012). Isolation and identification of a novel polysaccharide-peptide complex with antioxidant, anti-proliferative and hypoglycaemic activities from the *abalone* mushroom. Biosci. Rep., 32(3): 221-228.
- Lung, M.Y. and Tsai, J. C. (2009): Antioxidant properties of polysaccharides from the *Willow Bracket* medicinal mushroom, *Phellinus igniarius* (L.) Quel. (*Aphyllorphoromycetideae*) in submerged culture. Int. J. Med. Mushrooms., 11: 383-394.
- Nour EL-Dein, M.M.; Elfallal, A.A.; Toson, E.A. and Herecher, F.E. (2004). Exopolysaccharides

- production by *Pleurotus pulmonarius*. Pkistan J. Biol. Sci., 7(6): 1078-1084.
- Osman, M.; Ahmed, W.; Hussein, F. and El-sayed, H. (2014). Endopolysaccharides production and growth of *Flammulina velutipes* 6 under Submerged Conditions. J. Chem. Bio. Phy. Sci. Sec. B., 4:3350-3366.
- Rahar, S.; Swami,G.; Nagpal, N.; Nagpal, M.A. and Singh,G.S. (2011). Preparation, characterization, and biological properties of β -glucans. J. Adv. Pharm. Tech. Res., 2 (2): 94-103.
- Raut, J.K. (2019). Current Status, Challenges and Prospects of Mushroom Industry in Nepal. Int. J. Agric. Econ., 4(4): 154-160.
- Sharma, S.K.; Gautam, N. and Atri, N.S. (2015). Optimization, Composition, and Antioxidant Activities of Exo-and Intracellular Polysaccharides in Submerged Culture of *Cordyceps gracilis* (Grev.) Durieu & Mont. Evidence-Based Comple. and Alter. Med., 2015:1- 8.
- Shen, J.W.; Shi, C.W. and Xu, C.P. (2013). Exopolysaccharides from *Pleurotus pulmonarius* Fermentation Optimization, Characterization and Antioxidant Activity. Food Technol. Biotechnol., 51(4): 520-527.
- Soares, A.A.; De Souza, C.G.M.; Daniel, F.M.; Ferrari,G.P.; Da Costa, S.M.G. and Peralta, R.M. (2009). Antioxidant activity and total phenolic content of *Agaricus brasiliensis* (*Agaricus blazei* Murril) in two stages of maturity. Food Chem., 112(4): 775- 781
- Ul-Haq, I.; Ullah, N.; Bibi, G.; Kanwal, S.; Sheeraz Ahmad, M. and Mirza, B. (2012). Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. Iran. J. Pharm. Res., 11(1): 241-249.
- Valverde, M.E.; Hernandez-Perez,T. and Paredes-Lopez, O. (2018). Edible Mushrooms: Improving Human Health and Promoting Quality Life. Int. J. Microbiol. Res., 2015: 1-14 .
- Vetter, J. (2007). Chitin content of cultivated mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*. Food Chem., 102: 6-9.
- Wu, Y.C.; Liang, Z. C.; Lu, C. P. and Wu, S. H. (2008). Effect of Carbon and Nitrogen Sources on the Production and Carbohydrate Composition of Exopolysaccharide by

- Submerged Culture of *Pleurotus citrinopileatus*. J. Food Dr. Anal., 16: 61-67.
- Zhao, X.; Sun, H.; Hou, A.; Zhao, Q.; Wei, T. and Xin, W. (2005). Ant ioxidant properties of two gallotannins isolated from the leaves of *Pistacia weinmannifolia*. Biochim. Biophys. Acta., 1725(1): 103-110.

الظروف المثلى وتوصيف السكريات عديدات التسكر الداخلية من فطر البلوروتس اورنجي وتطبيقاتها الممكنة

علاء مصطفى أبو زيد*، إيمان حسن فتحى عبد الظاهر*، هبة الله محمد
عبد*، أميرة أحمد أحمد مصطفى*

* قسم النبات و الفطريات ، كلية العلوم، جامعة طنطا، مصر

** قسم علم الحيوان، كلية العلوم ، جامعة الاسكندرية، مصر

أظهرت الدراسة ان زراعة فطر البلوروتس اورنجي على وسط غذائي يحتوى على البطاطس والدكستروز لمدة (12) يوم كفترة تحضين عند درجة 30 درجة سليوزية و استخدام 3 قرص بقطر 1 سم من الفطر أعطت أعلى إنتاجية من السكريات عديدات التسكر المستخرجة داخليا. وأوضحت النتائج بعد التحليل باستخدام جهاز HPLC أن عديدات التسكر الداخلية المستخلصة من فطر البلوروتس اورنجي يتكون من السكريات بيتا-جلوكان ، كيتين ، جالاكتان ، المانان بتركيزات 36,20% ، 22,60% ، 21,60% ، 19,60% . أثبتت الدراسة ان السكريات عديدات التسكر الداخلية المستخلصة من فطر البلوروتس اورنجي لها نشاط مضاد للأكسدة بنسبة 72,4% عند 0.8 ملليجرام/مل .