

COMPILATION OF FULL PAPERS

INTERNATIONAL CONGRESS OF THE MALAYSIAN SOCIETY FOR MICROBIOLOGY 2015 (ICMSM2015)

MICROBIOLOGY MEETING THE NEEDS OF A CHANGING WORLD



7-10 DECEMBER 2015
BAYVIEW BEACH RESORT, PENANG, MALAYSIA

Organised by



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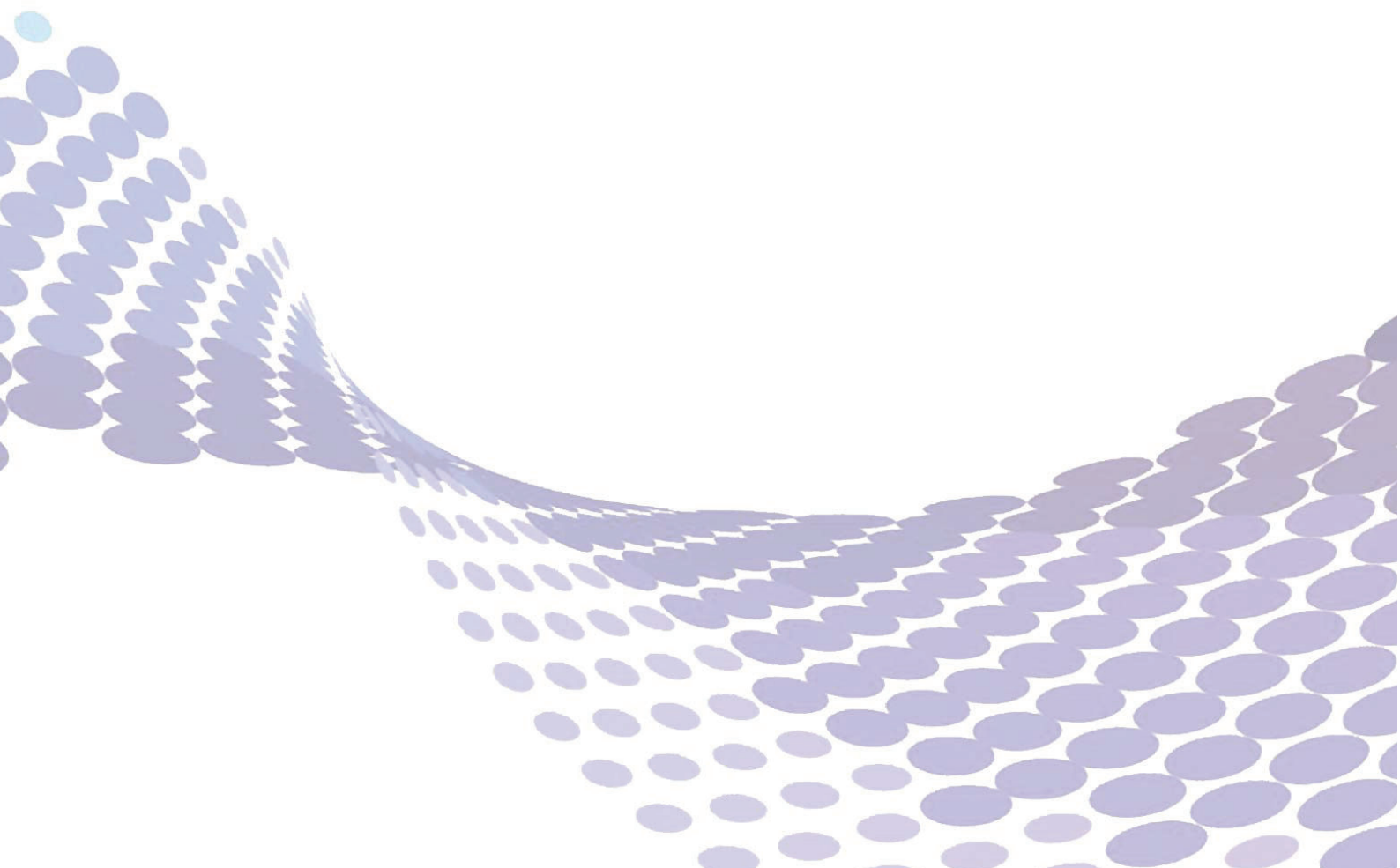
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FOREWORD
Director General of Higher Education
Ministry of Higher Education

I would like to take this opportunity to thank and congratulate the Malaysian Society for Microbiology (MSM) for organising International Congress of the Malaysian Society for Microbiology (ICMSM2015) themed 'Microbiology Meeting the Needs of a Changing World'. This is the fourth congress since its inception in 2009 and it endorses MSM's commitment to research, science and technology, knowledge sharing and global networking. These are key initiatives strongly supported by the Ministry of Higher Education (MOHE) that can lead to the promotion and contribution of microbiology to, not only national development, but also regional and international development. ICMSM2015 provides an opportunity for both national and international microbiologists in all aspects of microbiology to listen to leaders in the field, interact and forge networks in current topics of microbiology that may answer the many challenges that humans face.

I hope this gathering of microbiologists discussing ideas, techniques and findings in microbiology will further explore and understand the role of microbes in all aspects of applications including in agriculture, health, industries and the environment. In agriculture for example, the focus today is understanding how the microbiomes of soil and plants can play a role in enhancing yields without poisoning the soil with hazardous pesticides. These practices can ensure the food on our table is free from harmful residual effects of chemicals used in current food production. Further, is our brain function affected by our gut health? Understanding the gut microbiome may also throw light on several ailments afflicting humankind. These are pertinent challenges and from the programme for the next two and half days, I gather experts will share their expertise in these aspects in keynote and plenary lectures as well as in workshops. I wish the deliberation will be a fruitful one and all of you will return home enriched not only in microbiological knowledge but also gain a network of microbiologists to enhance your research activities.

I wish a very warm 'Selamat Datang' (welcome) to the foreign participants to Malaysia and hope that your stay here is a pleasant and memorable one

Prof. Dato Dr. Asma Ismail



FOREWORD
President
Malaysian Society for Microbiology

Assalamualaikum W.B.T. and Greetings!

On behalf of the Malaysian Society for Microbiology (MSM), I would like to warmly welcome you to the 4th International Congress of the Malaysian Society for Microbiology (ICMSM2015). It is our hope that this biennial congress will provide the avenue for the exchange of leading edge research in the field of microbiology and the time to renew old friendships and make new acquaintances.

In recent years, scientific tools have become available for in-depth studies on microbes and the complex microbial communities in which they live. Advanced technologies used today in microbiology such as the 'omics' and the recent genome editing approach will greatly expand our ability to discover and benefit from microbial capabilities. These approaches will shed light on the myriad capabilities of microbes and their communities, hence generate knowledge of microbial interactions and uniqueness such that they can be harnessed to improve human health, food security, energy production and environmental sustainability.

Thus, we hope that the theme of this symposium, 'Microbiology meeting the needs of a changing world' would pave the way for deliberations on the advancement of microbiology in the face of global challenges. The Organising Committee has put together an exciting scientific programme to accommodate the various areas of microbiology and set the stage for pertinent discourse in-line with the theme.

I am also proud to announce that this year's Microbiology Award recipient is Professor Dr. Thong Kwai Lin from the University of Malaya. Her immense contribution to Microbiology in Malaysia, specifically in the studies of molecular microbiology of food borne and nosocomial bacterial pathogens is lauded.

I would like to extend my utmost gratitude to the Organising Committee members for tirelessly working together and supporting this event. I would also like to convey my deepest gratitude to our sponsors for their continued support towards the MSM. A sincere appreciation also goes to the guests of honour, invited speakers and participants for making this event a success.

With this, I bid you a rewarding time of deliberation and a very pleasant stay in Penang.

Assoc. Prof. Dr. Farah Diba Abu Bakar



**FOREWORD
Chairperson
Organising Committee ICMSM2015**

On behalf of the Organising Committee, I extend a warm welcome to all participants to the ICMSM2015 themed 'Microbiology Meeting the Needs of the Changing World' which has attracted 306 participants from 10 countries. Four keynote lectures, 10 plenary lectures, 27 contributed oral and 213 poster presentations cover the full spectrum of key issues in microbiology today. This will be a platform for microbiologists to exchange views and ideas and to establish collaborative and / or strategic partnerships. It provides a unique opportunity for the younger fraternity in pursuit of excellence in microbiological research to interact with experts in the various fields. I take this opportunity to thank all invited speakers for sparing time to be with us at ICMSM2015 over the next couple of days sharing knowledge which we believe will have an impact on the future directions in microbiological research to address various challenges of the humankind.

We are happy to have received encouraging support from all institutions involved in the organisation of ICMSM2015. The organising team has been working tirelessly to ensure the congress a success. I record my thanks for all their time, suggestions commitment and dedication to make ICMSM 2015 a fruitful congress. We record special thanks to our partners in science – our sponsors and supporters for their continued support which has enabled us to give all participants an exciting mixture of classical and modern trends such as microbiome in microbiology research.

Prof. Dr. Vikineswary Sabaratnam

PREFACE

The International Congress of the Malaysian Society for Microbiology 2015 (ICMSM2015) that is held at the Bayview Beach Resort, Penang from the 7-10 December 2015 is an exceptional showcase of the broad disciplines encompassed within the diverse specialities of the field of Microbiology. This biennial Congress acts as a platform to showcase both local and international expertise, research and knowledge sharing. The knowledge garnered through basic fundamental research all the way to exploratory in-depth research serves to enlighten us on the extraordinary capabilities of Microbes in line with our theme for the Congress, 'Microbiology meeting the needs of a changing world'. A total of 24 full papers that were presented orally or as posters at the Congress by local and international participants have been compiled from the seven concurrent sessions (Agricultural Microbiology, Environmental Microbiology, Food Microbiology, General Microbiology, Industrial Microbiology, Medical Microbiology and Microbial Omics and Molecular Biology). The Scientific Committee of ICMSM2015 has reviewed these papers. We hope that these short research papers will contribute towards knowledge as well as provide links for collaboration and networking.

The American Society for Microbiology will also be contributing towards the Student Poster and Oral Presentation Awards, which will be based on the quality of the presentations made at this Congress. In addition, the Scientific Committee also invites submissions of research papers or short communications from participants for consideration in the Malaysian Journal of Microbiology, a Scopus Indexed journal. There will be a Special Issue published between June-July 2016 from manuscripts submitted for consideration from abstracts presented at ICMSM2015.

I would like to take this opportunity to thank members of the Scientific Committee for their contribution in time and effort in making this compilation possible. Special thanks also goes to Mr. Jonathan Ling for technical services rendered in putting together this publication.

Prof. Dr. Kalaivani Nadarajah
Head Scientific Committee ICMSM2015

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PHYSIOLOGICAL STUDIES ON MYCELIAL BIOMASS GROWTH OF *Cunninghamella* sp FROM SARAWAK, MALAYSIA

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ABSTRACT

Cunninghamella belongs to the zygomycetes group of microfungi. Biomass of *Cunninghamella* can be effectively used for many biotechnological applications such as bio-transformation of drugs, bioremediation and enzyme production. Physiological studies on how carbon and nitrogen nutrition sources including pH, light and temperature influence the mycelial biomass growth of *Cunninghamella* sp were investigated. *Cunninghamella* sp was grown on liquid basal medium of 30 ml supplemented separately with various carbon and nitrogen nutrients in conical flasks for 15 days after which its mycelial biomass yield were measured. *Cunninghamella* sp was able to produce good mycelial biomass growth on monosaccharides, dissacharides and complex carbon sources with no significant differences between them. Ammonium tartrate, as an inorganic nitrogen nutrition significantly produced the highest biomass compared to other nitrogen nutrients used. *Cunninghamella* sp was able to grow well under different light regimes, neutral pH to acidic medium and at temperature range of 20 to 35 °C with very low growth at extreme low and high temperatures of 5 °C and 40 °C. The results from this study indicate the ability of *Cunninghamella* sp to grow well under a wide range of conditions, an ability of the zygomycetes which is deemed responsible for their ubiquitous nature and fast growth. *Cunninghamella* sp showed specific preferences for nitrogen nutrition and temperature when grown for its biomass production.

Keywords: *Cunninghamella*, biomass, physiological requirements, microfungi, basal medium.

INTRODUCTION

The physiology of an organism is key to its functionality under different environmental conditions which is particularly important for biotechnological applications. Physiology of a fungus entails its nutrition, growth and metabolism in both living and non-living environments (Walker & White, 2011). Fungi are easy to grow and produce high yield of biomass which can be directly efficient for the removal of waste (Park *et al.*, 2005) and serve as inoculum for other uses. *Cunninghamella* sp. belongs to the class of fungi Zygomycetes which have been particularly useful in the bioremediation and removal of harmful chemical compounds from the environment such as anthracene (Guiraud *et al.*, 2008), phenanthrene (Cerniglia & Yang, 1984), fluoranthene (Pothuluri *et al.*, 1992), tributyltin chloride produced by fossil fuel combustion and distillation (Bernat & Długoński, 2007); and removal of dyes, salts and surfactants from wastewaters and effluents (Tigini *et al.*, 2010), as well as breakdown and metabolism of naphthalene (Cerniglia & Gibson, 1978) and polycyclic aromatic hydrocarbons (PAHs) (Banerjee, 2013). Most reports on the physiology of *Cunninghamella* sp. did not study for its mycelial biomass on different classes of carbon and nitrogen nutrient sources

utilizable by this fungus, as well as different light regime, temperature and pH. Previous studies are mostly done under toxic conditions, either for bioremediation, drug transformation or metabolism (Alves *et al.*, 2015; El-Morsy, 2004; Tigini *et al.*, 2011). However, the basic physiological conditions that can affect large scale production and use of *Cunninghamella* biomass before being subjected to adverse conditions have not been carried out. Higher mycelial production of *Cunninghamella* sp. will increase its efficiency for the removal of waste and biotransformation of chemical compounds and serve as inoculum for other biotechnological uses. This present work was aimed to investigate the biomass production of *Cunninghamella* sp. under different conditions of carbon nutrition, nitrogen nutrition, pH, temperature and light.

MATERIALS AND METHODS

Isolation of *Cunninghamella* sp. and preparation of basal medium

Cunninghamella sp. was isolated from green leaf of *Macaranga* sp during a microfungus survey from Kubah National Park in Sarawak. The isolates of *Cunninghamella* sp. were maintained at 27 °C (room temperature) on Potato dextrose agar (PDA) with subsequent sub-cultures until ready for use.

For mycelial biomass yield test of *Cunninghamella* under different physiological conditions, a standard basal medium was used which was adjusted for each physiological test and consisted of 20.00 g glucose, 2.00 g yeast, 0.30 g KH₂PO₄, 0.30 g MgSO₄, 0.20 g CaCl₂, 0.20 mg FeSO₄, 0.10 mg CuSO₄, 0.5 mg pyridoxine, 0.5 mg niacin and 0.3 mg ZnSO₄ (Mandyam *et al.*, 2010; Troncozo *et al.*, 2015) with slight modifications.

Effect of different carbon source on biomass yield

The various carbon nutrients representing various classes of carbon (Table 1.) were supplemented in the basal medium by replacing the glucose at the same quantity (i.e. 20g/L). The liquid without any carbon source served as the control. 30 mL of the basal medium was dispensed into 150 mL conical flasks, pH regulated to 6.5 and was covered for autoclaving at 121 °C for 15 min. After cooling, each conical flask was inoculated with mycelia disc of 5.0 mm diameter from the pure cultures of *Cunninghamella* sp. isolates and incubated on bench top at room temperature for 15 days (Troncozo *et al.*, 2015), each treatment with three replicates.

Table 1. Different classes of carbon and nitrogen nutrition sources evaluated in this study

	CARBON NUTRIENT	CLASSIFICATION
1.	Glucose	Monosaccharide
2.	Lactose	Oligosaccharide
3.	Malt extract	Polysaccharide
4.	Starch	Polysaccharide
5.	Glycerol	Sugar alcohol (Polyol)
6.	Sucrose	Oligosaccharide (Polyol)
7.	Myo-inositol	Sugar alcohol
	NITROGEN NUTRIENT	
8.	Ammonium tartrate	Inorganic nitrogen
9.	Ammonium nitrate	Inorganic nitrogen
10.	Ammonium sulphate	Inorganic nitrogen
11.	Ammonium acetate	Inorganic nitrogen
12.	Sodium nitrate	Inorganic nitrogen
13.	Peptone	Complex organic nitrogen
14.	Yeast Extract	Complex organic nitrogen

After 15 days, the mycelium in each conical flask was harvested by shaking the bottle to make sure attached mycelium are released, filtered through a pre-weighed 9 cm diameter Whatman No. 1 filter paper, oven- dried at 60 °C for 3 days and weighed (Gbolagade *et al.*, 2006).

Effect of different nitrogen on biomass yield

For the nitrogen nutrients (Table 1.), the basal medium was supplemented with the various nitrogen replacing the yeast in the basal medium and the same procedure was followed as for the carbon sources.

Effect of different pH on biomass yield

For pH, 30 mL of the basal medium was dispensed into the conical flasks with the pH adjusted to values of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 9.0 and 10.0 with 1 N NaOH or 1 N HCl before autoclave (Hasan Rreza Etebarian *et al.*, 2012; Luangharn *et al.*, 2014). Each pH value with three replicates, and the same growth procedure was followed as for the carbon source.

Effect of different light regime on biomass yield.

Three different light regimes, constant darkness, constant light and alternate light and darkness were used. For all the experimental set-ups, after 15 days, the mycelium were harvested and weighed as described above.

Effect of different temperature on biomass yield

For the temperature requirements for mycelial biomass production of *Cunninghamella* sp., 30 mL of the basal medium with glucose as the carbon source was dispensed into 150 mL conical flasks and was processed as done for carbon nutrition. After inoculating the flasks with the mycelial disc of the isolates, the conical flasks were incubated at eight different temperatures; 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C for 15 days under static conditions. Each treatment with three replicates.

Data Analysis

The means of each treatment were analysed by Analysis of Variance (ANOVA) in SPSS v.22 and the mean values were evaluated using Duncan multiple range test (DMRT) at significant value of $p > 0.05$.

RESULTS AND DISCUSSION

The results of biomass yield of *Cunninghamella* sp. are presented in Figure 1 and Table 2. All the tested carbon nutrient sources were favourable for mycelial biomass growth of *Cunninghamella* sp. except the control without any carbon nutrient (Figure 1). Fungi in the class Zygomycetes, of which *Cunninghamella* belongs, are known as sugar fungi (Fukasawa *et al.*, 2011; Hudson, 1968; Shanthi & Vittal, 2010) and grow very fast on any substrate due to their complex ability to produce different enzymes that can breakdown complex sugars and utilize the simple sugars.

Ammonium tartrate yielded the highest biomass growth (230.00 mg $\pm$ 0.00) of *Cunninghamella* sp. which is significantly different from that of all other nitrogen sources tested. Ammonium acetate (186.67 mg $\pm$ 6.67) and ammonium sulphate (173.33 mg $\pm$ 3.33) were also favourable for mycelial biomass while the control yielded the least (50.00 mg $\pm$ 10.00). Preference of ammonium tartrate by *Cunninghamella* sp. can be attributed to the fact that ammonium tartrate is a weak ammonium salt, thus conversion of the salt into ammonia first before being transported into the fungus metabolism leaving behind hydrogen ions (Tong & Rajendra, 1992), did not acidify the medium to levels unfavourable for the growth of

Cunninghamella sp. This result suggests that without any nitrogen source in the growth substrate, *Cunninghamella* sp. will still grow but slowly. This further elaborates on the growth of members of the Zygomycetes even under low nutrient. This result is in line with the report by Sukrutha *et al.* (2014) about the growth of *Cunninghamella* on a wide range of nitrogen sources which include ammonium sulphate, ammonium chloride, sodium nitrate, potassium nitrate and ammonium nitrate include sodium nitrate.

At pH 3, 4, 5, 8, 9 and 10, there was low growth of *Cunninghamella* sp., but at pH 6 and 7, the mycelial biomass growth was high and not significantly higher than each other. From this result, pH 7 gave the highest biomass yield which was significantly higher than those obtained at other pH values. This follows the trend which reported by Montenegro Stamford *et al.*, (2007) on *Cunninghamella elegans* at pH 7.

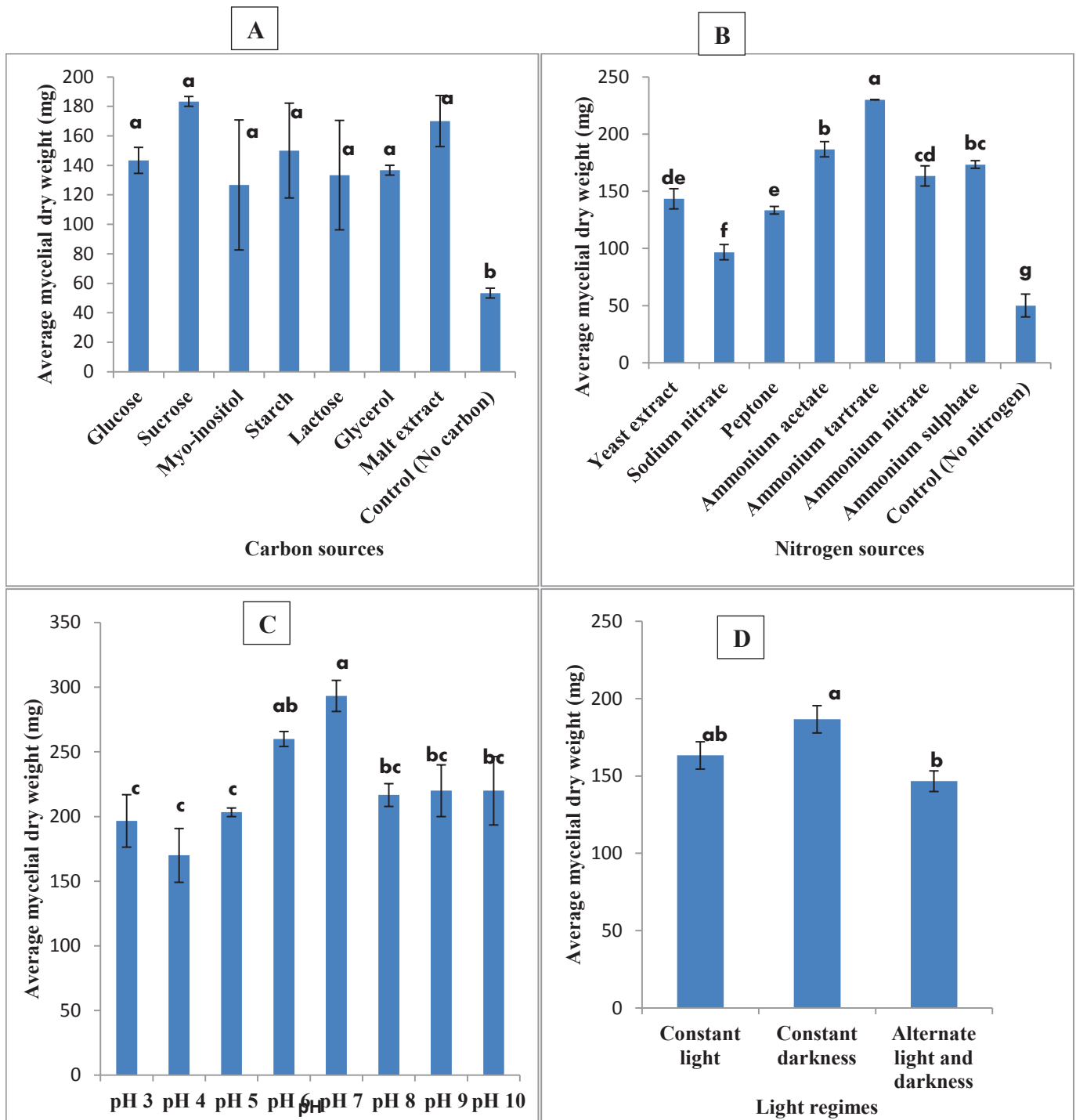
Constant darkness was better than alternate light and darkness, but not significantly different from constant light (Figure 1). Kwon-Chung and Bennett (1992) reported a similar observation that members of the Zygomycetes generally do not require light exposure for their growth.

Cunninghamella sp. grew well at 20 and 25 °C with biomass yield of 186.67 ± 8.82 mg and 193.33 ± 12.02 mg, respectively (Table 2). The growth obtained at 20 and 25 °C was significantly higher ($p > 0.05$) than that obtained at all the other temperature used in this study. Low mycelial biomass growth was similarly obtained at 5 °C (70.00 ± 15.28 mg), 10 °C (83.33 ± 8.82 mg) and 40 °C (73.33 ± 3.33 mg), with a moderate growth at 35 °C (133.33 ± 16.67 mg). Zygomycetes generally have a wide range of temperature for growth. *Mucor circinelloides*, for example, cannot grow at temperatures above 39 °C (Reinhardt *et al.*, 1970), on the other hand, *Cunninghamella bertholletiae* can grow at temperature of 45 °C (Weitzman & Crist, 1980). Thus, the observed moderate temperature required by *Cunninghamella* sp. in this study can be an isolate-dependent factor.

Table 2. Effect of temperature on mycelial biomass yield of *Cunninghamella* sp.

Temperature (°C)	Average Mycelial dry weight after 15 days $\pm$ S.E.M
	<i>Cunninghamella</i> sp.
5	70.00 ± 15.28^c
10	83.33 ± 8.82^c
15	176.67 ± 3.33^{ab}
20	186.67 ± 8.82^a
25	193.33 ± 12.02^a
30	173.33 ± 32.83^{ab}
35	133.33 ± 16.67^b
40	73.33 ± 3.33^c

Means with same alphabet down the columns are not significantly different from each other ($p \leq 0.05$). Duncan Multiple range test (DMRT).



REFERENCES

Bernat, P. & Długosz, J. (2007). Tributyltin chloride interactions with fatty acids composition and degradation ability of the filamentous fungus *Cunninghamella elegans*. *Int Biodeterior Biodegrad* 60, 133–136.

Cerniglia, C. E. & Gibson, D. T. (1978). Metabolism of naphthalene by cell extracts of *Cunninghamella elegans*. *Arch Biochem Biophys* 186, 121–127.

Cerniglia, C. E. & Yang, S. K. (1984). Stereoselective metabolism of anthracene and phenanthrene by the fungus *Cunninghamella elegans*. *Appl Environ Microbiol* 47, 119–124.

El-Morsy, E. M. (2004). *Cunninghamella echinulata* a new biosorbent of metal ions from polluted water in Egypt. *Mycologia* 96, 1183–1189.

Fukasawa, Y., Osono, T. & Takeda, H. (2011). Wood decomposing abilities of diverse lignicolous fungi on nondecayed and decayed beech wood. *Mycologia* 103, 474–482.

Gbolagade, S., Sobowale, A. & Adejoye, D. (2006). Effect of Environmental and Nutritional Factors on Mycelial Biomass Yield of Ten Wild Nigerian Mushrooms during Cellulase and Amylase Production. *Afr J Biotechnol* 5, 1464–1469.

Guiraud, P., Bonnet, J. L., Boumendjel, A., Kadri-Dakir, M., Dusser, M., Bohatier, J. & Steiman, R. (2008). Involvement of *Tetrahymena pyriformis* and selected fungi in the elimination of anthracene, and toxicity assessment of the biotransformation products. *Ecotoxicol Environ Saf* 69, 296–305.

Hasan Rreza Etebarian, Nvazallh Sahebany & Mehrnoush Mohammadifar, M. H. (2012). Effect of cultural condition on biomass production of some Nematophagous fungi as biological control agent. Egypt. Acad. J. Biolog. Sci.

Hudson, H. J. (1968). The ecology of fungi on plant remains above the soil. *New Phytol* 67, 837–874.

Luangharn, T., Karunarathna, S. C., Hyde, K. D. & Chukeatirote, E. (2014). Optimal conditions of mycelia growth of *Laetiporus sulphureus* sensu lato. *Mycology* 5, 221–227.

Mandyam, K., Loughin, T. & Jumpponen, A. (2010). Isolation and morphological and metabolic characterization of common endophytes in annually burned tallgrass prairie. *Mycologia* 102, 813–821.

Montenegro Stamford, T. C., Montenegro Stamford, T. L., Pereira Stamford, N., De Barros Neto, B. & De Campos-Takaki, G. M. (2007). Growth of *Cunninghamella elegans* UCP 542 and production of chitin and chitosan using yam bean medium. *Electron J Biotechnol* 10.

Park, D., Yun, Y.-S. & Park, J. M. (2005). Use of dead fungal biomass for the detoxification of hexavalent chromium: screening and kinetics. *Process Biochem* 40, 2559–2565.

Pothuluri, J. V., Heflich, R. H., Fu, P. P. & Cerniglia, C. E. (1992). Fungal metabolism and detoxification of fluoranthene. *Appl Environ Microbiol* 58, 937–941.

Reinhardt, D. J., Kaplan, W. & Ajello, L. (1970). Experimental Cerebral Zygomycosis in Alloxan-Diabetic Rabbits I. Relationship of Temperature Tolerance of Selected Zygomycetes to Pathogenicity. *Infect Immun* 2, 404.

Shanthi, S. & Vittal, B. P. R. (2010). Fungi associated with decomposing leaf litter of cashew (*Anacardium occidentale*). *Mycology* 1, 121–129.

Sukrutha, S. K., Adamechova, Z., Rachana, K., Savitha, J. & Certik, M. (2014). Optimization of Physiological Growth Conditions for Maximal Gamma-linolenic Acid Production by *Cunninghamella blakesleeana*-JSK2. *J Am Oil Chem Soc* 91, 1507–1513.

Tigini, V., Prigione, V., Donelli, I., Freddi, G., Bertolotto, A., Varese, G. C. & S.A, P.-E. (2010). *Fungal biomass preparation for the treatment of industrial wastewaters containing pollutants*.

Tigini, V., Prigione, V., Donelli, I., Anastasi, A., Freddi, G., Giansanti, P., Mangiavillano, A. & Varese, G. C. (2011). *Cunninghamella elegans* biomass optimisation for textile wastewater biosorption treatment: an analytical and ecotoxicological approach. *Appl Microbiol Biotechnol* 90, 343–352.

Tong, C. C. & Rajendra, K. (1992). Effect of Carbon and Nitrogen Sources on the Growth and Production of Cellulase Enzymes of a Newly Isolated *Aspergillus* sp. *Pertanika* 15, 45–50.

Troncozo, M. I., Gómez, R. P., Arambarri, A. M., Balatti, P. A., Bucsinszky, A. M. M. & Saparrat, M. C. N. (2015). Growth and oxidative enzymatic activity of in-vitro cultures of *Ciliobuxia* buxifolia. *Mycoscience* 56, 58–65.

Walker, G. M. & White, N. A. (2011). Introduction to Fungal Physiology. In *Fungi Biol Appl*, pp. 1–35. John Wiley & Sons, Ltd.

Weitzman, I. & Crist, M. Y. (1980). Studies with clinical isolates of *Cunninghamella* II. Physiological and morphological studies. *Mycologia* 72, 661–669.