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Effects of various carbon and nitrogen sources on mycelial biomass production of *Macrolepiota procera* and *Polyporus squamosus* in submerged culture

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ABSTRACT
This study was carried out to investigate the effects of various carbon and nitrogen sources on mycelial biomass production of *Macrolepiota procera* and *Polyporus squamosus*, important edible mushrooms in Turkey, in submerged cultures. Seven carbon (dextrose, glucose, lactose, maltose, mannitol, sucrose and xylose) and six nitrogen sources ($(NH4)_2HPO_4$, NH_4NO_3, $Ca(NO3)_2$, malt extract, peptone and yeast extract) were used in the study. The Sabouroud (SB) and Dextrose Yeast Extract (DYE) liquid media without nitrogen and carbon were considered as the control. All carbon and nitrogen sources promoted significantly (p<0.01) mycelial biomass production in *M. procera* and *P. squamosus*. The optimum carbon and nitrogen sources for mycelial biomass production showed changes depending on mushroom species. As a result, the most suitable carbon source for mycelial biomass production in *M. procera* was dextrose and followed by glucose, mannitol, sucrose and lactose, while the medium with xylose was in *P. squamosus*. Peptone and malt extract as a nitrogen source in *M. procera* gave the best result for the biomass production, whereas yeast extract was the most favorable nitrogen source in *P. squamosus*. The lowest mycelial biomass production for the both mushroom species was determined in the control medium without carbon and nitrogen. In conclusion, the basic informations obtained from this study could be useful in the optimization of submerged culture conditions and nutritional requirements for mycelial biomass production in *M. procera* and *P. squamosus*.

Keywords:
Carbon
Macrolepiota procera
Mycelial biomass
Nitrogen
Polyporus squamosus
Submerged culture

Değişik karbon ve azot kaynaklarının *Macrolepiota procera ve Polyporus squamosus* mantarlarının sıvı kültürde misel biyomas üretimi üzerine etkileri

ÖZET
Bu çalışma, değişik karbon ve azot kaynaklarının Türkiye'nin önemli yenilebilir mantarlarından *Macrolepiota procera* ve *Polyporus squamosus*'un sıvı kültürde misel biyomas üretimi üzerine etkisini araştırmak için yapılmıştır. Çalışmada yedi karbon (dekstroz, glikoz, laktoz, maltoz, mannitol, sükroz ve ksiloz) ve altı azot kaynağı ($(NH_4)_2HPO_4$, NH_4NO_3, $Ca(NO_3)_2$, malt ekstrakt, pepton ve maya ekstrakt) kullanılmıştır. Azot ve karbon içermeyen Sabouroud (SB) ve Dekstoz Maya Ekstrakt (DYE) sıvı ortamları kontrol olarak kabul edilmiştir. Tüm karbon ve azot kaynakları *M. procera* ve *P. squamosus'*da misel biyomas üretimini önemli (p<0.01) derecede teşvik etmiştir. Misel biyomas üretimi için optimum karbon ve azot kaynakları, mantar türlerine bağlı olarak değişiklik göstermiştir. Sonuç olarak, misel biyomas üretimi için *P. squamosus*'da en uygun karbon kaynağı ksiloz iken, *M. procera* için dekstroz bulunmuş, bunu glikoz, mannitol, sükroz ve laktoz izlemiştir. Misel biyomas üretimi için azot kaynağı olarak *M. procera'*da pepton ve malt ekstrakt, *P. squamosus'*da ise maya ekstrakt en iyi sonucu vermiştir. Her iki mantar türünde de, en düşük misel biyomas üretimi karbon ve azot içermeyen kontrol ortamında belirlenmiştir. Bu çalışmadan elde edilen temel bilgilerin *M. procera* ve *P. squamosus'*da misel biyomas üretimi için besin gereksinimleri ve sıvı kültür koşullarının optimizasyonunda faydalı olabileceği düşünülmüştür.

Anahtar Sözcükler:
Karbon
Macrolepiota procera
Misel biyoması
Azot
Polyporus squamosus
Sıvı kültür

1. Introduction

Edible mushrooms are considered as one of the alternative food sources to meet the nutritional requirements of increasing world population because they are good sources of proteins, minerals, vitamins and fibre (Chang and Miles, 2004). Their usage has been increasing day by day due to their significant roles in human health and nutrition (Hassan and Medany, 2012).

Traditionally, some mushrooms have been produced on the solid substrate using composts or lignocellulosic wastes such as straw or wood, however this process usually takes several months to obtain fruiting bodies depending on species and substrate, requires intensive labor and the control of production conditions is difficult (Zhang et al., 2011). Submerged culture of mushrooms is widely used for efficient production of mycelial biomass, polysaccharides, enzymes, metabolites, proteins and vitamins from mushrooms (Park et al., 2001; Fang and Zhong, 2002). When compared to the cultivation on solid media, submerged culture has many advantages such as higher mycelial biomass production at a small area and in a shorter time with lesser chances of contamination, achievement of fungal biomass with high and consistent quality, its low cost, the feasibility of mass production in a compact space and year around production (Yang et al., 2003; Tang et al., 2007; Wu et al., 2008). Hence, a great deal of attention has been focused recently on mycelial biomass production in submerged culture because it is a rapid and promising alternative cultivation method for obtaining fungal biomass (Yang and Liau, 1998; Kwon et al., 2009). Mycelial biomass production in submerged culture has significant industrial potential and it can be used in the formulation of nutraceuticals and functional foods (Lee et al., 2004). Mycelial biomass is an important source of dietary fiber, protein, bioactive compounds and it is also free of cholesterol. Morever, mycelial biomass can be easily digested (Moore and Chiu, 2001). The mycelial biomass production in submerged culture depends on many factors such as culture medium, temperature, pH, carbon and nitrogen sources, C/N ratio, minerals (Shu et al., 2004). Therefore, it is essential to optimize the culture conditions and nutritional requirements for mycelial biomass production.

Macrolepiota procera (Scop. ex Fr.) Singer, an edible saprophytic mushroom, belongs Lepiotaceae family. It is commonly called the Parasol Mushroom. *M. procera* is widely distributed and consumed in South-East Asia, Europe, North Africa and North America (Vellinga et al., 2003). It is relatively rich in terms of protein, carbohydrate, minerals, vitamins, dietary fiber and has also low fat content and good medicinal value (Falandysz et al., 2008; Kuldo et al., 2014; Kumari and Atri, 2014). This mushroom has delicate texture, good taste, pleasant smell and excellent quality, but only its cap can be used because its stem is very fibrous, tough and inedible. The fruiting bodies of this mushroom occure during late summer and autumn.

Polyporus squamosus (Huds.) Fr. is an edible saprophytic or parasitic mushroom belonging to the Polyporaceae family. It is called dryad's saddle mushroom or pheasant's back mushroom and characterized by innumerous pores under the cap. It has a widespread distribution in North America, Australia, Asia and Europe. The fruiting bodies of *P. squamosus* occur on living and dead hardwood trees during spring and autumn. Young fruiting bodies are soft, but toughened with age. Therefore, young specimens due to good flavour and taste are preferred for eating. It was reported that *P. squamosus* had good nutritional and medicinal value (Ertan and Gülyavuz, 1991; Elmastas et al., 2007).

M. procera and *P. squamosus* are fairly common in the Black Sea Region of Turkey with mild and rainy climate. These two wild edible mushroom species are widely consumed by the peoples, and sold at the local markets in the Black Sea Region due to their economic importance (Pekşen et al., 2008). Because, there is no commercial cultivation of these two mushrooms in Turkey, they are only collected from nature during the fructification seasons.

Although many studies carried out on the effects of various nutrient sources for mycelial biomass production of different mushroom species in submerged culture (Kim et al., 2003; 2005; Gbolagade, 2006; Gbolagade et al., 2006a, b; Huang et al., 2007; Zhou et al., 2009a, b; Li et al., 2010; Manjunathan and Kaviyarasan, 2011; Hassan et al., 2012; Lai et al., 2014; Ramesh et al., 2014), the researches on determination of optimal culture conditions and nutritional requirements for mycelial biomass production of *M. procera* and *P. squamosus* in submerged culture are inadequate. Thus, the objective of this study was to determine the effects of different carbon and nitrogen sources on mycelial biomass production (mycelial dry weight) of *M. procera* and *P. squamosus* in submerged culture.

2. Materials and Methods

2.1. Preparation of sporocarp isolation

The fruiting bodies of *M. procera* and *P. squamosus* were collected from a mixed deciduous forest at the campus of Ondokuz Mayıs University,

Samsun, Turkey during autumn season in 2008. Mushroom species were conventionally identified (Phillips, 1994). Pure mycelial cultures of *M. procera* and *P. squamosus* were obtained from mushroom tissues using the method described by Jonathan and Fasidi (2003). For this purpose, surface sterilized small pieces of fruiting bodies were aseptically transferred on Malt Extract Agar (MEA) medium and the cultures were incubated at 25 °C in complete darkness. Stock cultures, stored at 4 °C, were subcultured every 3 months and used as inoculum for the further investigations.

2.2. Effect of carbon and nitrogen sources on mycelial biomass production

To determine the most suitable carbon and nitrogen sources for the mycelial biomass production of *M. procera* and *P. squamosus*, seven carbon and six nitrogen sources were tested. This experiment was performed on the Sabouroud (SB) medium (40 g/l glucose and 10 g/l peptone) in *M. procera* and on Dextrose Yeast Extract (DYE) medium (30 g/l dextrose and 3 g/l yeast extract) in *P. squamosus*. In DYE liquid medium prepared for *M. procera,* dextrose, glucose, mannitol, maltose, sucrose (saccharose), lactose and xylose were used as carbon sources. Each of the carbon sources for *M. procera* was added individually to the SB medium instead of glucose at the concentration of 40 g/l and mixed throughly. Each of carbon sources for *P. squamosus* was supplemented individually to the DYE medium instead of dextrose at the rate of 30 g/l. SB and DYE media without any carbon were used as the control in the experiments (Kadiri and Fasidi, 1994). Malt extract, peptone, yeast extract, $(NH_4)_2HPO_4$, NH_4NO_3 and $Ca(NO_3)_2$ were used as nitrogen sources in the study. In *M. procera*, each of nitrogen sources was individually added to the SB medium instead of peptone at the concentration of 10 g/l and mixed throughly, while they were added individually to the DYE medium instead of yeast extract at the rate of 3 g/l in *P. squamosus*. SB and DYE media without nitrogen served as the control (Kadiri and Fasidi, 1994). The initial pH values of the liquid media prepared with various carbon and nitrogen sources were measured. 50 ml of liquid media were dispensed into 100 ml erlenmeyer flasks. The mouth of each flask was sealed with a cotton plug and covered with aluminium foil. Then, the media were sterilized by autoclaving at 121 ^{o}C for 20 min. After cooling, each flask was aseptically inoculated with 2 mycelial discs 0.5 cm in diameter cut from actively growing mycelial cultures of *M. procera* and *P. squamosus*. After that, the inoculated flasks were incubated at 23 °C for 14 days in *M. procera* and at 25 °C for 8 days in *P. squamosus* under dark conditions on a rotary shaker incubator at 100 rpm. The experiments were arranged in a Completely Randomized Design (CRD) with 4 replications.

In order to determine mycelial biomass production, the mycelial pellets in each flask were harvested by filtration through a pre-weighted Whatman No. 2 filter paper at the end of incubation period and washed several times with distilled water. Then, the obtained mycelia were dried in an oven at 70°C for 24 h to a constant weight and the mycelial dry weight was measured to determine mycelial biomass (Joshi et al., 2013). The mycelial biomass yield was expressed as g/l. The final pH values in the resulting culture filtrates were also recorded immediately after harvesting.

2.3. Analysis of data

Data from the present study were subjected to ANOVA using SPSS program (version 10.0) and results were expressed as mean values. Means were compared by Duncan's multiple range test. Square root transformation was applied to data on mycelial biomass of *P. squamosus* in various carbon sources prior to statistical analyses as no mycelial growth was observed in control medium.

3. Results and Discussion

ANOVA results revealed that there were significant differences ($p<0.01$) among carbon sources for mycelial biomass production of *M. procera* in submerged culture (Table 1). All carbon sources promoted mycelial biomass production of *M. procera*. The highest mycelial biomass (7.62 g/l) was recorded in the medium containing dextrose which was not statistically different from media containing glucose, mannitol, sucrose and lactose. Media with maltose and xylose supported moderate biomass production in *M. procera*. The lowest mycelial biomass (1.52 g/l) was determined in control medium without carbon due to lack of carbon in the growth medium.

In general, mycelia of many mushrooms can grow over a wide range of carbon source (Yang et al., 2003). In this study, dextrose was the best carbon source for *M. procera*. It shows that this mushroom produces enzymes utilizing dextrose better than other carbon sources. Carbohydrate utilization ability of an organism depends on type of enzyme produced by the organism. Our results were similar with Kim et al. (2003), Adejoye et al. (2006), Zhou et al. (2009a) and Manjunathan and Kaviyarasan (2011) who stated that dextrose was the most suitable carbon source for mycelial biomass production of *Cordyceps militaris*, *Pleurotus florida*, *Laccocephalum mylittae* and *Lentinus tuberregium*, respectively. The second best carbohydrate for this mushroom was glucose. It is

Table 1. Effect of carbon sources on mycelial biomass production of *M. procera* in submerged culture

Carbon sources	Mycelial biomass (g/l)[1]	Initial pH	Final pH[1]
Dextrose	7.62a**	6.68 ns	6.28d**
Glucose	5.82ab	6.70	6.38cd
Lactose	4.58abc	6.75	6.28d
Maltose	4.07bc	6.80	6.45bc
Mannitol	4.88abc	6.83	6.69a
Sucrose	4.72abc	6.77	6.41bcd
Xylose	3.41bc	6.55	5.34e
Control	1.52c	6.85	6.53b

**Significant at 0.01 level (values indicated with the same letters within the same column are not significantly different), ns: non significant, [1]Mycelial biomass and final pH value were measured at the end of the 14th day of incubation at 23°C.

suggested that mannose and glucose were the best carbon compounds for the biomass production of *Lepiota procera* (Gbolagade, 2006). The similar results on the utilization of glucose by some mushrooms has been reported (Jonathan and Fasidi, 2001; Xu et al., 2003; Joo et al., 2004; Lee et al., 2004; Kim et al., 2005; Gbolagade et al., 2006b; Shih et al., 2008; Zhou et al., 2009b; Li et al., 2010; Kibar and Pekşen, 2011; Hassan and Medany, 2012; Hassan et al., 2012; Pekşen et al., 2013; Lai et al., 2014; Ramesh et al., 2014). Wei et al. (2008) stated that glucose was the best candidate as the carbon source because of its easy to use and low cost compared to the other carbon sources. Result of the present study was also compatible with the report of Griffin (1994) who suggested that glucose, mannitol and fructose are the most commonly utilised sugars for mushrooms. Likewise to our study, the lower mycelial biomas was obtained with xylose compared to other carbon sources in *Paecilomyces tenuipes* (Xu et al., 2003), *Cordyceps sinensis* (Dong and Yao, 2005), *Agrocybe cylindracea* (Kim et al., 2005) and *Lignosus rhinocerus* (Lai et al., 2014).

The initial pH values of the liquid media prepared with various carbon sources ranged from 6.55 to 6.85. No significant differences were found among initial pH values. The final pH values of the filtrates at the end of incubation period were found between 5.34 and 6.69. The lowest final pH was determined in xylose medium while the highest final pH was observed in mannitol medium. After mycelial growth in submerged culture, the final pH values decreased in all of the carbon sources.

Final pH values of the liquid media containing different carbon sources were consistent with the results of Adejoye et al. (2006). However, our final pH results were higher than the values reported by Xu et al. (2003), Kim et al. (2003), Gbolagade (2006) and were lower than that of Gbolagade et al. (2006a). Similarly, the lowest final pH was determined in xylose (Joo et al., 2004) and the highest final pH was observed in mannitol (Gbolagade et al., 2006a) among the carbon sources.

The effect of nitrogen sources on mycelial biomass production of *M. procera* was significant (p<0.01) (Table 2).

Among the nitrogen sources, the maximum mycelial biomass production was found in the medium containing peptone (9.40 g/l), and malt extract (7.01

Table 2. Effect of nitrogen sources on mycelial biomass production of *M. procera* in submerged culture

Nitrogen sources	Mycelial biomass (g/l)[1]	Initial pH	Final pH[1]
$(NH_4)_2HPO_4$	2.60b**	7.74ns	6.33a**
NH_4NO_3	3.34b	5.21	3.71f
$Ca(NO_3)_2$	3.32b	5.65	3.56f
Malt extract	7.01a	5.30	4.59d
Peptone	9.40a	6.69	5.47c
Yeast extract	3.09b	6.29	6.03b
Control	1.72b	6.27	4.20e

**Significant at 0.01 level (values indicated with the same letters within the same column are not significantly different), ns: non significant, [1]Mycelial biomass and final pH value were me asured at the end of the 14th day of incubation at 23°C.

g/l). The minimum mycelial biomass production (1.72 g/l) was determined in the control medium. Yeast extract, $(NH_4)_2HPO_4$, $Ca(NO_3)_2$ and NH_4NO_3 had similar effect on mycelial biomass production of *M. procera* when compared with the control medium.

Nitrogen plays an important role in fungal growth, metabolite production and synthesis of enzymes (Kim et al., 2005). Lin and Yang (2006) reported that the effect of nitrogen source on the mycelial growth depended on species, culture media and growth conditions. The promoting of mycelial growth by peptone may be due to its carbon and amino acid composition (Garraway and Evans, 1984). The highest mycelial biomass in submerged culture for *Cordyceps sinensis*, *Pleurotus florida, Auricularia polytricha*, *Hirsutella* sp. and *Pleurotus ostreatus* was obtained from the medium containing peptone (Dong and Yao, 2005; Adejoye et al., 2006; Jonathan et al., 2009; Li et al., 2010; Nwokoye et al., 2010. Jonathan and Fasidi (2001) stated that malt extract supported significant mycelial biomass production in *Psathyerella atroumbonata*. In the present study, organic nitrogen sources (peptone, malt extract and yeast extract) gave better results than inorganic nitrogen sources ($(NH_4)_2HPO_4$, NH_4NO_3 and $Ca(NO_3)_2$). Shih et al. (2006) suggested that most of basiomycetes prefer organic nitrogen sources for their growth in submerged cultures rather than inorganic form. Organic nitrogen sources are absorbed by the cells easier than the inorganic ones (Kang et al., 1997). Our result was in accordance with the results of Yang et al. (2003), Joo et al. (2004), Manjunathan and Kaviyarasan (2011) and Joshi et al. (2013) who reported that organic nitrogen sources gave relatively higher mycelial biomass in liquid cultures when compared with the inorganic nitrogen sources. The lowest mycelial biomass among the nitrogen sources for various macrofungi was obtained from ammonium phosphate, ammonium nitrate, calcium nitrate (Kim et al., 2003, Xu et al., 2003; Joo et al., 2004; Lee et al., 2004; Huang et al., 2007; Li et al., 2010; Manjunathan and Kaviyarasan, 2011). This could be attributed to nitrate ions inhibited mycelial growth of some basidiomycetes (Griffin, 1994). However, the findings obtained from the present study were contrary to those of Lai et al. (2014) and Ramesh et al. (2014) who stated that calcium nitrate and ammonium nitrate were the most suitable nitrogen source for the mycelial biomass production of *Lignosus rhinocerus* and *Xylaria* sp., respectively.

The initial pH values of the media prepared with various nitrogen sources did not show statistically significance. The highest final pH (6.33) was recorded in the medium containing $(NH_4)_2HPO_4$, while the lowest final pH values were obtained when $Ca(NO_3)_2$ and NH_4NO_3 were used as the nitrogen source (3.56 and 3.71, respectively). At the end of incubation period, pH values determined in all media containing different nitrogen sources were decreased compared to initial pH values.

Kim et al., (2003) found that the highest final pH was in $(NH_4)_2HPO_4$, while the lowest final pH was recorded in NH_4NO_3 (Joo et al., 2004) and $Ca(NO_3)_2$ (Adejoye et al., 2006; Gbolagade, 2006). The final pH values determined for nitrogen sources in the present study were higher than the values reported by Xu et al. (2003) and Joo et al. (2004), but lower than that reported by some other researchers (Jonathan and Fasidi, 2001; Adejoye et al., 2006; Gbolagade, 2006; Gbolagade et al., 2006a).

The carbon sources significantly stimulated the mycelial biomass production in *P. squamosus* ($p<0.01$) (Table 3). The best carbon source was found to be xylose for the mycelial biomass production with 3.30 g/l. The media containing glucose, dextrose and maltose also supported good biomass production of *P. squamosus*, but their promotive effect on biomass

Table 3. Effect of carbon sources on mycelial biomass production of *P. squamosus* in submerged culture

Carbon sources	Mycelial biomass (g/l)[1]	Initial pH	Final pH[1]
Dextrose	1.70b**	6.38 ns	4.12e**
Glucose	1.80b	6.41	4.50d
Lactose	0.90cd	6.38	8.42a
Maltose	1.50bc	6.39	8.47a
Mannitol	0.70d	6.40	7.87b
Sucrose	0.70d	6.47	8.41a
Xylose	3.30a	6.41	4.71c
Control	0.00e	6.41	8.26a

**Significant at 0.01 level (values indicated with the same letters within the same column are not significantly different),
ns: non significant, [1]Mycelial biomass and final pH value were measured at the end of the 8th day of incubation at 25°C. Square root transformation was applied to data on mycelial biomass prior to statistical analyses.

production was lower than xylose. Mycelial biomass drastically decreased when sucrose, mannitol and lactose were used as carbon source. No mycelial growth was found in control medium.

The different carbon sources might have different effects on mycelial growth of mushrooms. The highest mycelial biomass of *Schizophyllum commune* was found in the medium containing xylose by Joshi et al. (2013). Jonathan and Fasidi (2001), Lee et al. (2004), Adejoye et al. (2006), Gbolagade et al. (2006a, b) and Shih et al. (2008) determined that glucose, dextrose and maltose supported good biomass production in submerged cultures of different mushroom species. Mycelial biomass production of *Sarcodon aspratus* and *Lepiota procera* was poor in the medium containing sucrose (Joo et al., 2004; Gbolagade, 2006), while the low mycelial biomass of *Agrocybe cylindracea* was found in the medium with mannitol (Kim et al., 2005).

There was no statistically significant difference among the initial pH values of the media. The final pH values of the filtrates ranged from 4.12 (dextrose) to 8.47 (maltose). At the end of 8th day of incubation, final pH values of the media with dextrose, glucose and xylose were decreased when compared to initial pH values of the media. The highest final pH among the carbon sources was observed in maltose (Jonathan and Fasidi, 2001), sucrose (Joo et al., 2004) and lactose (Kim et al., 2003; Xu et al., 2003). In this study, similar results were found regarding final pH of media.

It was found that nitrogen sources had significant (p<0.01) effect on mycelial biomass production of *P. squamosus* (Table 4). Yeast extract medium produced the highest mycelial biomass (3.50 g/l), whereas nitrogen free control medium had the lowest mycelial biomass (0.40 g/l) due to limited nutrition in the growth media. Peptone, malt extract and NH_4NO_3 media followed by the yeast extract for the good biomass production of *P. squamosus*. In the $(NH_4)_2HPO_4$ and $Ca(NO_3)_2$ media, mycelial biomass production significantly decreased when compared with the other nitrogen sources.

Table 4. Effect of nitrogen sources on mycelial biomass production of *P. squamosus* in submerged culture

Nitrogen sources	Mycelial biomass (g/l)[1]	Initial pH	Final pH[1]
$(NH_4)_2HPO_4$	0.80c**	7.97^{ns}	6.73a**
NH_4NO_3	1.70b	6.84	3.15e
$Ca(NO_3)_2$	1.00c	6.78	4.43c
Malt extract	1.80b	6.39	3.37de
Peptone	1.90b	6.88	3.84d
Yeast extract	3.50a	6.65	5.09b
Control	0.40d	7.13	4.70bc

**Significant at 0.01 level (values indicated with the same letters within the same column are not significantly different), ns: non significant, [1]Mycelial biomass and final pH value were measured at the end of the 8th day of incubation at 25°C.

The stimulatory effect of yeast extract on mycelial biomass yield may be linked with its high carbohydrates, amino acids and vitamins composition (Gbolagade et al., 2006b). In submerged culture, yeast extract contributed to the highest mycelial biomass production in many fungal species (Jonathan and Fasidi, 2001; Joo et al., 2004; Lee et al., 2004; Gbolagade et al., 2006a, b; Huang et al., 2007; Zhou et al., 2009a; Manjunathan and Kaviyarasan, 2011; Hassan and Medany, 2012; Hassan et al., 2012; Joshi et al., 2013; Ramesh et al., 2014). As in the *M. procera*, organic nitrogen sources were more suitable for the mycelial biomass production of *P. squamosus* compared to inorganic nitrogen sources. This was similar to the findings of Joo et al. (2004) in *Sarcodon aspratus*, Manjunathan and Kaviyarasan (2011) in *Lentinus tuberregium* and Joshi et al. (2013) in *Schizophyllum commune*.

Initial pH values of the media varied between 6.39 and 7.97 did not show differences. Conversely, significant differences were found among the final pH values of the media. Among the nitrogen sources, the highest final pH (6.73) was determined in the $(NH_4)_2HPO_4$ while the lowest final pH (3.15) was obtained from the NH_4NO_3. After mycelial growth in submerged culture, the final pH values decreased in all of the nitrogen sources.

The final pH values in the present study were lower than those of Jonathan and Fasidi (2001), Gbolagade (2006) and Gbolagade et al. (2006a). The highest and lowest final pH values were found in $(NH_4)_2HPO_4$ and NH_4NO_3, respectively, by Kim et al. (2003) and Joo et al. (2004).

4. Conclusions

It was concluded that various carbon and nitrogen sources significantly influenced and promoted mycelial biomass production of *M. procera* and *P. squamosus*.

Study results also indicated that the most suitable carbon and nitrogen sources for mycelial biomass production may be varied with the mushroom species. The most suitable carbon sources for mycelial biomass production in *M. procera* were dextrose, glucose, mannitol, sucrose and lactose, while the medium with xylose was the best in *P. squamosus*. Peptone and malt extract were the most suitable nitrogen sources for mycelial biomass production of *M. procera*, whereas the highest mycelial biomass in *P. squamosus* was achieved with yeast extract. Thus, the present study provided useful information on the effects of carbon and nitrogen sources added in the growth media for improving of mycelial biomass production in *M. procera* and *P. squamosus*. However, further studies on the effect of macro and micro elements, carbon and nitrogen ratio, vitamins, amino acids, phytohormones, culture media, optimum concentrations of nutrients and incubation conditions on efficient production of mycelial biomass in the submerged culture of these mushroom species should be conducted.

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