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RESEARCH ARTICLE

OPTIMIZATION OF SUBMERGED CULTURE CONDITIONS FOR THE ENHANCED PRODUCTION OF MYCELIAL BIOMASS OF *PLEUROTUS FLABELLATUS*

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ABSTRACT

The study investigates *Pleurotus* sp is potential growth in producing mycelium and enhancing biomass production for their stimulatory effects under submerged culture condition. Addition of 25.07 gL^{-1}at the 21st day's stage of exponential growth stationary phase give the most excellent stimulatory effect on growth of mycelium and fungal biomass production increase. The decline phase on 28th days under submerged culture biomass of dry weight production (20.50gL^{-1}) level decrease. The optimized nutritional parameters were influencing the mycelia growth biomass productions enhance on carbon source in the form of glucose (23.71gL^{-1}) at 21st days under submerged condition. Peptone (23.73gL^{-1}) was found good nitrogen source for higher biomass production and stimulates the fungal growth. The effects of carbon/nitrogen (C/N) ratios were identified high yield biomass production on Glucose: Yeast Extract (G: YE) at 21st days (23.22gL^{-1}). Phosphorus (P) form of Monosodium phosphate (NaH_2PO_4) and Potassium (K) in the form of Pottassium chloride(KCl) their involving process (P: 25.31gL^{-1}; K: 22.79 gL^{-1}) also change the growth and yield of biomass production between stationary phase and decline phase. The Magnesium (Mg) and Calcium (Ca) sources (Mg: 23.14 gL^{-1};Ca: 23.01gL^{-1}) also specific changes and maximal mycelial growth as well as biomass production. The optimal media comparison of the environmental factors, Temperature at 30°C (26.22gL^{-1}), pH 5.5 (23.22gL^{-1}) and light at dark condition (24.34gL^{-1}) under submerged culture for mycelial growth and production of biomass respectively. The potential use of mycelium growth and biomass production processes for can be achieve the future generations without human disorder disease from fungal metabolites.

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INTRODUCTION

Medicinal and wild mushrooms have been widely used as a tonic food and herb remedies in China, Japan, and other Asian countries since ancient times (Wasser 2002). Mushrooms were widely used for medicinal purposes in China, Japan and other Asian countries from ancient times (Wasser, 2002). These macro fungi have potential pharmacological and biological activities (Lee 1996; Kiho 1993) and contain many alkaloids, phenolic compounds and lectin like proteins with anti-tumor (Sone *et al*., 1985), immuno-stimulating (Yoshida *et al*., 1996)hypoglycemic activity (Yang *et al.*, 2000). The morphological properties of the fungal cell wall and EPS are main play on the submerged culture conditions they can also be excreted to the culture medium. Extensive studies have been carried out to obtain bioactive polysaccharides from many wild and medicinal mushrooms (Kim *et al*., 2002). To obtain bioactive molecules from *Pleurotus* spp. many investigators have spent their efforts cultivating medicinal or edible mushrooms on submerged culture condition rather than in submerged cultures (for mycelial extract and/or bioactive molecules production). Artificial culture condition obviously have the potential for higher mycelial production in a small scale and in particular time without contamination. In addition, exopolysaccharide which have more effects with submerged culture on biological properties can be simultaneously produced (Park *et al*., 2002). Around two thousands medicinal and edible fungi have been reported among which only 20 species are domesticated, of which 3 to 5 species are commercially available in the market (Kaviyarasan, 1992). The optimization process is very essential for the cultivation of

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good and high yield medicinal or edible mushrooms. The bioactive molecules of many edible and wild mushrooms are naturally formed in submerged cultures and use culture media containing mineral nutrients, vitamins and complex source such as yeast extract, peptone, malt extract and amino acids (Yang and Liau, 1998; Jonathan and Fasidi, 2001a,b; Park *et al*., 2001; Lin and Sung, 2006). *Pleurotus* is a Basidiomycetes fungus belonging to the class Agaricomycetes, and family Pleurotaceae. Submerged liquid culture and fruit body is reported to contain anti-tumor polysaccharides. These intra and extra polysaccharides (Jung *et al*., 2003) have been identified to have pharmaceutical and nutritional value (Morais *et al*., 2002). The bioactive molecules produced by *Pleurotus* sp., ß-1,3 glucans play a vital role as biological response difference (Bohn and BeMiller 1995) which stimulate the immune system of the host and exert an extensive range of immune pharmacological activities, in particular an antitumor effect and the inhibition of metastasis (Gunde and Cimerman, 1999). In the present investigation, the fungal growth physiology under submerged culture conditions at different incubation period, nutritionalvalue,growth factors and various environmentalfactors were optimized for the production of mycelial biomass dry weight of *Pleurotus flabellatus*.

MATERIALS AND METHODS

Microorganism and growth conditions

The seed culture of *Pleurotus flabellatus* (P7) was collected from Pachipaari forest. The P7 strain genomic DNA was isolated and nucleotide sequence submitted to Genbank the accession number KT970056. The strain P7 were maintained Fungal Culture collection 2 (FCC2), Centre for Advanced Studies in Botany, University of Madras, Chennai, India and used throughout this study. Seed culture was maintained on Potato Dextrose Agar (Hi-media) medium in slant. The slant seed culture was grown in a 250ml Erlenmeyer flask containing 100 ml of seed culture medium at 25 °C for 4thdayin dark condition (Bo-Bo Zhang *et al*., 2011). 14 day's old culture was inoculated with 10 % (v/v) of the seed medium. Stock seed culture was maintained in FCC 2 room at 25 °C.

Analytical methods

The flask containing PDA medium was autoclaved at 121°C for 15 min. Further, it was cool and the medium was inoculated with 14 day's seed culture and incubated at 25°C up to 28thday. The dry weight of biomass was measured after frequent washing with distilled water through a Whatman No.1 filter paper (Sigma Aldrich, Grade 1: 11 µm) and left overnight at 70°C. Samples collected for biomass were estimated at 7th, 14th, 21st and 28th day's from the culture flask for the study of various parameters.

Optimization of different parameters, onPleurotus flabellatus under submerged culture condition

Influence of incubation time in the mycelial growth

The *Pleurotus flabellatus* was inoculated at different incubation periods up to 28thday. Their growth, development stage along with the mycelia dry weight of biomass was also recorded at regular intervals.

Nutritional factors influencing the mycelial growth

The fungal mycelia were cultivated in PDB liquid media containing different carbon sources (1 % (w/v)) such as; glucose, fructose, maltose, cellulose, starch, sorbitol and lactose. To study the effect of various nitrogen sources such as Ammonium nitrate, Ammonium sulfate, Potassium nitrate, Peptone, Urea, Beef Extract, Sodium nitrate, Calcium nitrate and Yeast extract were given to the medium in the ratio of 0.5 % (w/v). Similarly, to find out the effect of carbon and nitrogen ratio (C/N), in the form of Glucose/Yeast extract (G/YE), Glucose/Sodium Nitrate (G/SN), Fructose/ Yeast extract (F/YE) and Fructose/ Sodium Nitrate (F/SN) was given by the ratio of 1 % (w/v) /0.5 % (w/v) on PDB liquid medium. The phosphorus viz. Monopotassium phosphate (KH_2PO_4), Dipotassium phosphate (K_2HPO_4), Potassium Dihydrogen phosphate (H_2KO_4P) and Monosodium phosphate (anhydrous) (NaH_2PO_4) were added to the mother medium in the ratio of 0.5 % (w/v) to determine the effect of phosphorus. Potassium chloride (KCl), Potassium sulfate (K_2SO_4), Potassium hydroxide (KOH) and Potassium carbonate (K_2CO_3) was added to the medium in the ratio of 0.5 % (w/v) to study the effect of Potassium. The influence of Magnesium on the medium was studied by using various Magnesium sources such as Magnesium oxide (MgO), Magnesium phosphate tribasic (Mg_3 (PO_4) $_2$), Magnesium hydroxide Mg (OH) $_2$, Magnesium chloride ($MgCl_2$) and Magnesium sulfate anhydrous ($MgSO_4$) in the ratio of 0.5 % (w/v). To supplement, calcium sources such as calcium chloride ($CaCl_2$), Calcium carbonate ($CaCO_3$), Calcium citrate ($Ca_3(C_6H_5O_7)_2$) and Monocalcium phosphate ($Ca(H_2PO_4)_2$) was grown in seed culture at the ratio of 0.5 % (w/v) in the medium.

Environmental factors influencing the mycelial growth

To examine the environmental effect on the development of the mycelia, pH and temperature adjustments were made. pH ranged from 5.5 to 9.5 and temperature was adjusted between 20°C to 40°C respectively. The light study was evaluated by four different light viz. Dark light, Near UV, Continuous day light and 12D;12L (Kbar B *et al*., 2008). The seed culture with Potato Dextrose Broth, were incubated for 21 days in dark at 25 ± 2°C under still condition.

RESULTS AND DISCUSSION

Incubation period factors influencing the mycelial growth of Pleurotus flabellatus

Effect of Incubation period

Different incubation periods were adopted to optimize maximum biomass production. On the 7thday (log phase) the biomass production of 5.0 ± 0.22 gL ^{1} was observed. Whereas, maximum (25.47 ± 0.40 gL ^{1}) biomass production was obtained on the 21st day (stationary phase). Subsequently decreased (20.40 ± 0.36 gL ^{1}) biomass production was observed on the 27th day. Concluding the growth and biomass increased from log phase to stationary phase (7th - 21st day) (Fig 1.) and reduced in the decline phase (21st - 28th day).The above findings were in accordance with Stinson *et al*., 2003.,

where the maximum growth rate and biomass production was observed in the stationary phase.

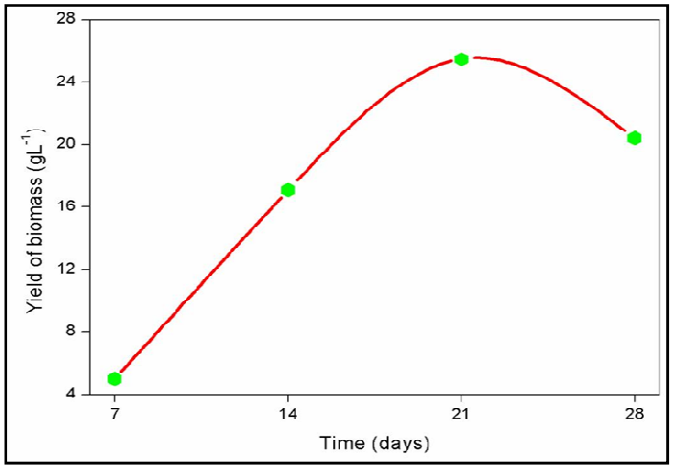

Figure 1.Effect of incubation period on *Pluerotus flabellatus* under submerged culture conditionfor twenty eight hours. The effect of different period were determined using twelve trials from 7, 14, 21 and 28th day incubation period (n=12). The results were expressed without error from n=12. The yield of biomass determined by using dry weight of each mycelium

Nutritional factors influencing the mycelial growth of P. flabellatus

Effect of carbon (C) source

To find a suitable carbon sources for mycelial biomass production under submerged condition, study was carried out in media containingthosedifferent carbohydrates sources (sugars), where each source was added to the PDB medium. Carbohydrate serves as an essential nutrition for the growth and development of macro-fungi (Xiao *et al*., 2006). Carbohydrate source in the form of glucoseshows increased the biomass production (24.13 ± 0.86 gL ^{1}) followed by fructose (23.06 ± 0.06 gL ^{1}). Similar results were observed in several other studies where glucose proved to be the best carbohydrate source in submerged culture of mushrooms (Xu *et al*., 2003; Nour El-Dein *et al*., 2004). In contrast, Yajie *et al*., 2002 reported that the best mycelial growth in *Ganoderma lucidum* was observed in the form of lactose followed by glucose and fructose. The influence of sorbitol (08.27 ± 0.16 gL ^{1}) on mycelial growth level was less significant when compared to other carbon source (**Table 1**).

Effect of nitrogen (N) source

Nitrogen is involved in physiological control and regulation of microorganism metabolism (Thomas *et al*., 1996). Hence nine kinds of organic and inorganic nitrogen sources on the mycelial biomass production were examined (**Table 2**).

Among the various nitrogen sources examined, highest mycelial growth was observed in peptone and yeast extracts (23.83 ± 1.13 and 24.41 ± 1.13 gL ^{1}. This may be probably due to the presence of protein, amino acid and other vitamin contents in the yeast extract. Similar results were observed in *Cordyceps jiangxiensis* (Xiao *et al*., 2006)and mycelial growth from *Lentinus subnudus* (Gbolagade 2006). However, low levels of biomass production were observed when urea was allowed in the growth medium. The macro fungus mainly depends on inorganic nitrogen sources (Kim *et al*., 2003) for optimum growth condition and physiological metabolism.

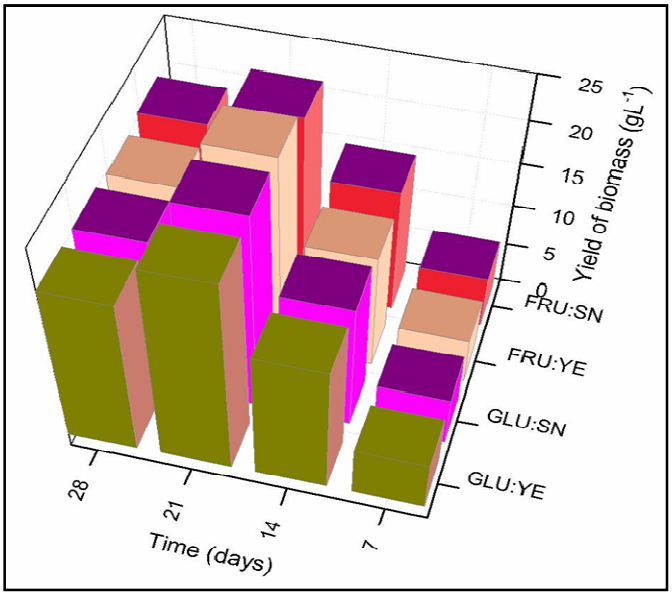

Figure 2. Yield of *Pluerotus flabellatus*in submerged culture condition after four different time intervals, 7, 14, 21 and 28th day respectively. The medium contain different carbon/ nitrogen ratio. Four different colors indicate four different C/N ratios with yield of biomass in dry weight in liter. Values are expressed without error and all experiments were conducted at three times for calculate error (n=3)

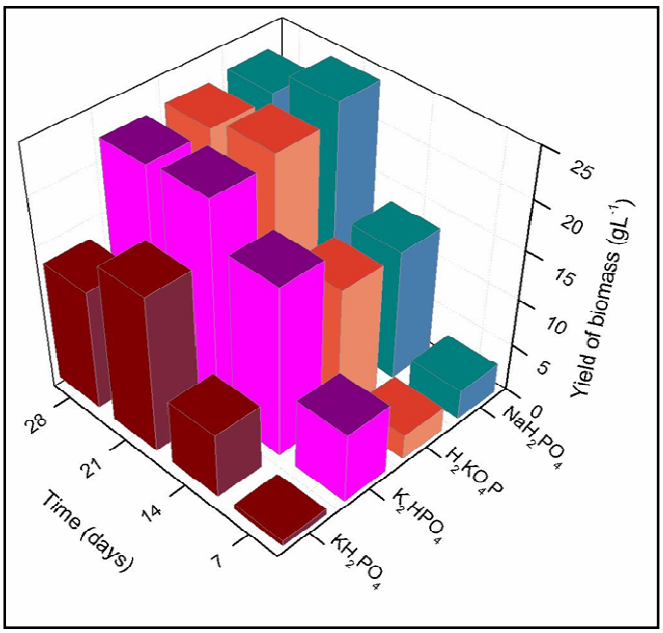

Figure 3. Phosphorus (P) source on *Pluerotus flabellatus* at different day's interval. The P four different sources were expressed at yield of biomass in liter. The time interval calculate three times in each experiment (n=3). Values are expressed without error bars from n=3. Each time intervals measure biomass of dry weight from n=12.

Nutritional factors influencing the Mycelial growth of *Pleurotus flabellatus*

Table 1. Effects of different carbon sources on mycelial growth of *P. flabellatus* under submerged culture condition. The values expressed from three different experiments. The values was determined with standarad errors (mean ± SD; n=3). Seven nitrogen source were determined from four different days intervals.Yield of biomass were calculate from four different time intervals (7, 14, 21, and 28 days)

Carbon source (gL^{-1})	Glucose	Fructose	Maltose	Cellulose	Starch	Sorbitol	Lactose
7thday	4.36±0.20	5.74±0.27	4.61±0.25	2.05±0.04	1.25±0.04	1.82±0.05	1.57±0.09
14thday	15.56±0.09	14.59±0.51	12.78±0.75	9.31±0.22	8.42±0.17	4.49±0.15	12.79±0.08
21stday	24.13±0.86	23.06±0.06	22.61±0.38	18.58±0.56	17.86±1.18	8.27±0.16	21.39±1.16
28thday	18.40±0.04	18.51±1.10	17.45±1.24	14.16±1.12	12.61±0.13	3.73±0.62	15.91±1.22

Table 2. Effects of different nitrogen sources on mycelial growth of *P. flabellatus* under submerged culture condition. The values expressed from three different experiments. The values was determined with standarad errors (mean ± SD; n=3). Yield of biomass were calculate from four different time intervals (7, 14, 21, and 28 days). Nine different nitrogen source were determined from four different days intervals

Nitrogen source (gL^{-1})	NH_3NO_3	NH_4SO_4	KNO_3	Peptone	Urea	Beef Extract	$NaNO_3$	$CaCO_3$	Yeast Extract
7th day	Ng	0.28±0.05	0.26±0.04	3.75±0.04	Ng	1.05±0.04	0.26±0.02	Ng	3.07±0.55
14th day	7.05±0.56	11.56±0.04	7.89±0.58	14.88±0.56	0.01±0.01	12.89±0.57	3.08±0.55	3.51±0.59	14.07±0.57
21st day	16.53±0.59	22.08±0.61	18.48±0.59	24.41±1.13	0.01±0.01	23.41±1.16	22.49±0.56	12.84±1.15	23.83±1.13
28th day	12.57±1.12	16.32±1.12	14.00±0.55	19.11±0.61	0.01±0	17.13±1.14	17.05±1.16	7.88±0.56	19.59±1.17

Ng – no growth

Effects of environmental factors

Table 3. Effects of Different pH on the mycelia growth of *P. flabellatus* under submerged culture condition. The values was expressed from three different experiments. The values was determined with standarad errors (mean ± SD; n=3). Yield of biomass were calculate from four different time intervals (7, 14, 21, and 28 days). Ten different pH range were determined from four different days intervals

pH	5.0	5.5	6	6.5	7	7.5	8	8.5	9	9.5
7th day	5.52±0.31	6.56±0.29	6.01±0.88	4.85±0.39	2.99±0.78	1.67±0.86	Ng	Ng	Ng	Ng
14th day	12.22±0.11	13.57±0.52	12.68±0.10	6.64±0.22	3.81±0.16	1.14±0.05	1.41±0.42	Ng	Ng	Ng
21st day	22.27±0.27	26.98±0.80	24.07±0.96	13.74±0.21	12.59±0.32	11.96±0.96	11.41±0.35	4.43±0.41	3.69±0.18	0.25±0.09
28th day	19.2±0.35	21.53±0.46	19.55±0.22	7.13±0.15	8.48±0.36	6.65±0.42	6.46±0.42	2.46±0.20	2.38±0.52	0.07±0.03

Ng – no growth

Table 4. Effects of Temperature on the mycelia growth of *P. flabellatus* under submerged culture condition. The values was expressed from three different experiments. The values was determined with standarad errors (mean ± SD; n=3). Yield of biomass were calculate from four different time intervals (7, 14, 21, and 28 days).Five different temperature range was determined from four different days intervals

Temperature °C	**20**	**25**	**30**	**35**	**40**
7th day	3.25±0.18	6.34±0.06	7.65±0.27	0.08±0.01	Ng
14thday	13.24±0.09	15.35±0.14	17.61±0.16	8.00±0.11	Ng
21st day	23.30±0.11	25.54±0.33	26.19±0.22	18.34±0.14	7.43±0.42
28th day	18.27±0.04	19.36±0.11	20.41±0.40	14.48±0.35	6.15±0.07

Ng – no growth

Table 5. Effects of Light on the mycelia growth of *P. flabellatus* under submerged culture condition. The values was expressed from three different experiments. The values was determined with standarad errors (mean ± SD; n=3). Yield of biomass were calculate from four different time intervals (7, 14, 21, and 28 days).Four different light intensity was determined from four different days intervals

	Dark	**Near UV**	**Continuous Day Light**	**12 Dark: 12 Light**
7th day	4.20±1.20	0.80±0.22	0.80±0.09	0.38±0.07
14thday	13.74±0.22	9.55±0.23	10.57±0.36	8.37±0.54
21st day	24.63±0.27	18.60±0.33	20.86±0.89	18.07±1.17
28th day	19.89±0.96	16.08±1.48	17.66±0.37	14.73±0.35

Effect of C/N ratio

Carbon / nitrogen ratio is an essential factor whichinvolved in metabolic changes, particularly in the growth of fungal mycelial production (**Fig. 2**). C/N in the form of G/YE in the ratio of 1 % (w/v): 0.5 % (w/v) ratio favored the growth of mycelial biomass production (23.60 ± 0.54 gL^{1}) followed by G/SN (23.83 ± 1.14 gL^{1}), Slightly decreased biomass production was observed in the form of F/YE (23.51 ± 0.60 gL^{1}). This may be due to the interference in the biosynthesis and growth, metabolism of the mycelial biomass produced. Carbon sources gave cellular secondary metabolic activity

(Hwang *et al*., 2003) while, nitrogen provided as protein for synthesis, amino acids and organic compounds (Kim *et al.,* 2005). In contrast, G/YE gave more yield than others.

Effect of phosphorus (P) source

Phosphates play a major role in growth differentiation. Among the tested phosphorus sources NaH_2PO_4 (24.52 ± 0.72 gL ^{1}) was the suitable source for the enhanced production of biomass. Effective production of biomass was also obtained in H_2KO_4P (22.92 ± 1.61 gL ^{1}) and K_2HPO_4 (22.06 ± 1.46 gL ^{1}) However, minimal biomass were observed in KH_2PO_4. The maximum fungal biomass of 24.52 ± 0.72 gL ^{1} (**Fig. 3**) observed in yeast extract supplemented medium. Lilly (1965) and Blumenthal, (1965) was reported, the P most abundant material in fungal hyphae while energy transfer to metabolism synthesis for buildup integral material of RNA, DNA, cofactors, coenzymes and phospholipds. In our study was more similar according past reporter. P in the form of NaH_2PO_4 was play in submerged culture condition.

Effect of Potassium (K) source

To study the effect of Potassium sources on biomass production, four different K sources were tested. The maximum biomass production of 22.90 ± 1.67 gL ^{1} was observed on KCl, followed by K_2SO_4 (22.42 ± 1.95 gL ^{1}) KOH (17.96 ± 1.47 gL ^{1}) and K_2CO_3 (13.54 ± 0.58 gL ^{1}) over a period of 21 days (**Fig. 4**). Complex of fungal hyphae absorbed K^+ ion use an transfer the electron for K^+ and H^+ redox pumb (Conway, 1953) while regulate the cellular osmotic pressure of the cell inside. The cation ions a play a vital role in activate coenzymes and other factors (Garraway & Evans, 1984).

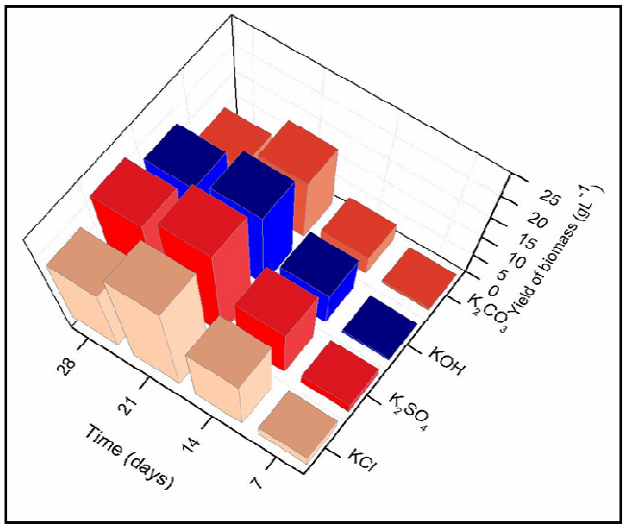

Figure 4. Effect of different Potassium (K) source on *Pluerotus flabellatus*. The growth of species was evaluated from dry weight of 7, 14, 21 and 28 days intervals. The values was expressed without error bars from triplicates (n=3). The yield of biomass was calculated based on dry weight in liter

Effect of Magnesium (Mg) source

Mg cofactor with some other enzymes mainly occurs in fungal cell walls and cell membrane. Presence of Mg sources, mainly affects the metabolites and the mechanism of biosynthesis (En-Shyh Lin *et al*., 2007). Mg^{+}ions act as ATP dependent a cofactor in enzymatic reactions while stabilizesthe cell membrane. These trace sources are influence to the production of other mushroom mycelia (Hwang *et al*., 2003; Kang *et al*., 1997; Jonathan and Fasidi, 2001). Effects of different Mg sources on the mycelial growth were studied in the medium. When Mg allowed in the form of $MgSO_4$ and $MgCl_2$ growth, biomass was increased as 22.96 ± 1.83 gL ^{1} and22.42 ± 0.87 gL ^{1} (**Fig. 5**) respectively. Similar results have been reported from *G. lucidum* (Hsieh *et al*., 2006). Interestingly, Mg in the form of Mg_3 $(PO_4)_2$ on liquid media decreased the biomass production.

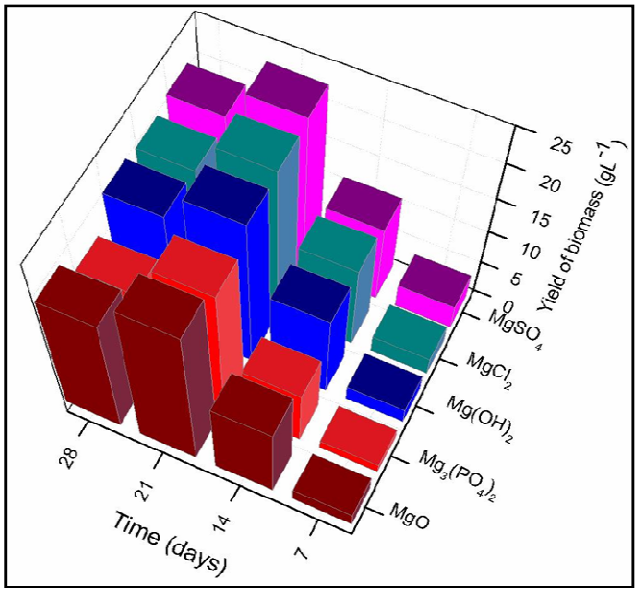

Figure 5. Effect of different Magnesium (Mg) source on *Pluerotus flabellatus* under submerged culture condition. Submerged culture biomass were determined from four different time intervals. The values are calculated from dry weight of *Pluerotus flabellatus*at particular days interval. The figure was indicates yield of dry weight in liter with without error bars. Each experiment were conducted triplicates (n=3)

Effect of Calcium (Ca) source

Enormous studies have been carried out to conclude that Calcium has a vital role in the tip development of fungal cell, and that Ca uptake is contained in the apex of the tip (Schmid *et al*., 1988; Gooday *et al*., 1990). Addition of calcium into the strainshow an efficient strategy to increase the yields of mycelial dry weight proved to be successful in fungal hyphae growth. The fungal biomass increased (22.45 ± 1.49 gL ^{1}) in the presence of $CaCO_3$ followed byCa_3 $(C_6H_5O_7)_7$ (22.31 ± 0.94 gL ^{1}) (**Fig. 6**).

Environmental factors influencing the mycelial growth of Pleurotus flabellatus

Effect of initial pH

The pH of the medium plays a major role in the nutrient uptake, but it is often an abandoned environmental factor (Kim *et al*., 2005).

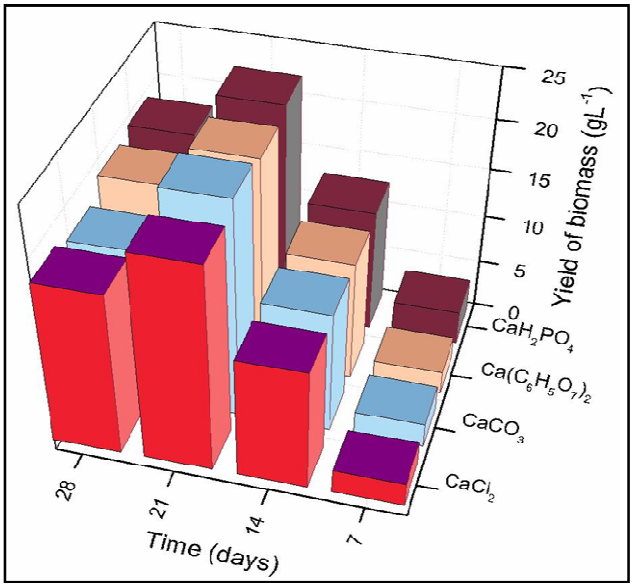

Figure 6.Effect of different Calcium (Ca) source on *Pluerotus flabellatus* under submerged culture condition. Submerged culture biomass were determined from four different time intervals 7, 14, 21 and 28th day respectively. The values are calculated from dry weight of *Pluerotus flabellatus* at particular days (7/14/21/28). The figure was indicates yield of dry weight in liter with without error bars. Each experiment were conducted triplicates (n=3)

To study the effect of pH on the growth of biomass, different pH was chosen, ranging from 5.0 to 9.5 at 0.5 pH interval (Table 3). The pH were affect lipid layer, growth differentiation, structure, the different nutrients factors, and biosynthesis (Shu and Lung 2004). Among the different pH studied maximum (26.98 ±0.80 gL ^{1}) Biomass were produced in the acidic range of pH 5.5 followed by pH 6.0 (24.07 ± 0.96 gL ^{1}). Mycelial biomass gradually decreased with the increase of pH. Several investigators claimed that the special morphology of fungi mycelia below the dissimilar initial pH value was the vital factor in biomass production and metabolite development (Wang *et al*., 1995; Shu and Lung 2004).This effect shows some information about varying the balance linking cell development and metabolic product. Many investigators find that different types of oyster mushroom growth was exponential at acidic nature for mycelial biomass production under the submerged culture (Kim *et al*., 2005) and the pH range was fixed biomass accumulation from metabolic changes (Wang and McNeil, 1995). Past researchers were reported, pH control the function, membrane stability, morphology of cells, structural component and upregulation of various nutrients (Shu and Lung, 2004).In the present investigation neutral pH and a little alkaline medium were suitable for the maximum production of mycelia.

Effect of Temperature

Suitable temperature for the maximum (26.19 ± 0.22 gL^{-1}) biomass production was recorded at 30 °C and was followed by 25.54 ± 0.33 gL^{-1} at 25 °C. As temperature increases, there was a reduction in the biomass production. At 40°C a reduced or no growth was observed on the 7th and 14th day and the maximum growth recorded was only 07.43 ± 0.42 gL^{-1}. Hence the present study proves that the ground temperature inhibit the cell development/growth of the fungus, this is probably due to the inhibition of cell development at higher temperatures (Table 4). The results were positive correlation from previous researchers, at 25-30°C maximum production of biomass from *A. cylindracea, P. tenuipes* (Kim *et al*., 2005; Chun *et al*., 2003).

Effect of Light

Light plays a major role in growth and differentiation of mycelia. The suitable light for the optimum production of biomass four different light forms were selected which includes dark light, near UV, continuous light, 12 hrs. light and 12 hrs. dark light. Among the above, increased (24.63 ± 0.27 gL^{-1}) biomass were obtained at dark light followed by continuous daylight (20.86 ± 0.89 gL^{-1}) (Table 5). The study deal with light and dark light regulation of fungal mycelia metabolism. The growth presence or absence of light source whether decrease or increase the growth rate in fungi (Carlile 1965). From Friedl *et al*., 2008a,b, mutant and wild type of *T.atroviride* was investigated light and darkness. Dark light was different growth rate than Light. From the phenomenon was support to the present studies while submerged culture maintained at dark conditions growth of yield increased.

Conclusion

The optimization of the growth conditions and different nutritional factors of mycelial biomass from *Pleurotus flabellatus* was investigated with appropriate statistical methodology. The present study also successfully elucidated that incubation hours, influence the fungal biomass along with nutritional and environmental factors. Further investigation is necessary to achieve large scale cultivation of mushroom.

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