



Studies on Optimization of Culture Conditions and Medium Components for the Production of Mycelial Biomass of *Auricularia delicata* under Submerged Fermentation

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Authors' contributions

This work was carried out in collaboration among all authors. Author MSJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LX, MFS and XA managed the analyses of the study. Author MSJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To optimize the culture conditions and medium components for the production of mycelial biomass of *A. delicata* under submerged fermentation.

Place and Duration of Study: China–Zambia Agricultural demonstration center and Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China between July 2019 and June 2020.

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Methodology: In this study, a single factor at a time method was employed in the optimization of submerged culture conditions and medium components for the production of mycelial biomass of *A. delicata* (strain YD 99). Each factor was screened independently while other factors were kept constant.

Results: The findings of this study demonstrate that the optimal culture conditions obtained were as follows: carbon source (Glucose) 20 g l⁻¹, pH 6.0, nitrogen source (Yeast extract) 2 g l⁻¹, mineral elements (K₂HPO₄+MgSO₄.7H₂O) 2g l⁻¹, and incubation temperature 25°C. The application of these optimal culture conditions produced a maximum concentration of 7.34g l⁻¹ mycelial biomass of *A. delicata*.

Conclusion: Consequently, our results indicated that the optimization of culture conditions and medium components is of significant importance for the cultivation of *A. delicata*.

Keywords: *Auricularia delicate*; mycelial biomass; submerged fermentation; optimal culture condition.

1. INTRODUCTION

Since ancient times varieties of mushrooms have been utilized globally as a nutritious resource and natural therapy [1-5]. Mushrooms contain potential nutrients, vitamins and minerals useful for the maintenance of health. Mushroom extracts possess the ability to trigger an immune response against some illness, thus promoting longevity [6-11]. Varieties of *Auricularia* mushrooms are reported to have exceptional properties including anticancer, antidiabetic, hypoglycemic, antioxidant, antimicrobial, antifungal, anti-inflammatory response and improved gastrointestinal activities [7, 12-14].

Previous studies revealed that *A. delicata* (Fr.) Henn was discovered by a German mycologist known as Paul Christoph Hennings in 1893 and aided its classification under the genus *Auricularia* [15, 16]. Despite its early discovery, the cultivation technology of this mushroom was not keenly investigated as compared to other *Auricularia* mushrooms. Recently investigators found that *A. delicata* (Fr.) Henn can be cultivated through both solid and liquid (submerged) fermentation [17-20]. These discoveries impelled its cultivation in various parts of the world, mainly Asia (China and Japan) and Africa (Zambia). Submerged fermentation is advantageous over solid fermentation due to its potential of producing a high yield of mycelial biomass with consistent quality, rapid growth and reduced chances of contamination, thus shortening the cultivation cycle [19,21-23]. Mycelial extracts of *A. delicata* contain various bioactive substances of industrial importance, with the use of submerged fermentation varieties of biological molecules including enzymes, polysaccharides, organic acids, flavours and nutritional supplements can be extracted from

high volumes of mycelial biomass [24-27]. Numerous studies show that the growth of mycelia is influenced by a number of factors that constitute the culture medium namely; pH, temperature, carbon source, nitrogen source, mineral elements whose effect on the production of mycelial biomass is noticeable [20,28,29].

Several researchers have successfully studied the cultivation conditions of *A. delicata* under solid medium, however, the cultivation conditions of *A. delicata* under submerged culture condition has not been clearly unveiled. Xing-Hong et al., [30] reported that the optimal cultivation conditions for *A. delicata* under solid medium include: temperature 28°C, pH 5.5-6.0, mineral elements (K₂HPO₄ and MgSO₄.7H₂O), sucrose and maltose as carbon sources, and yeast extract and peptone as nitrogen sources. Therefore, considering the significant benefits of submerged fermentation relative to solid fermentation, there is a need for unveiling the cultivation technology of *A. delicata* under submerged fermentation.

The purpose of this study consists of the optimization of culture conditions and medium components for the production of mycelial biomass of *A. delicata* with consistent quality. A single factor method was applied in screening each factor at a time to discover its effect on the production of mycelia biomass while other factors were kept constant.

2. MATERIALS AND METHODS

2.1 Fungal Strain

The strain of edible fungi *A. delicata* (YD99) was collected from Zambia and isolated in the laboratory of the Engineering Research Center of

Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University-China. The cultures were cultivated on a Potato Dextrose Agar (PDA) medium, incubated at 25°C for 7 days then stored in a refrigerator at 4°C.

2.2 Inoculum and Medium Preparation

The seed culture medium composed of glucose 20g l⁻¹, Yeast 2g l⁻¹, peptone 2g l⁻¹, 1g l⁻¹ MgSO₄·7H₂O, 0.5g l⁻¹ K₂HPO₄·3H₂O, and an initial pH value of 6.0. Four pieces of 5mm mycelia of *A. delicata* strain were transferred from 7 days old PDA plates to each 500ml Erlenmeyer flask containing 100ml of basal fermentation medium. Then each inoculated seed culture flask was incubated in a rotatory shaker at 120rev.min⁻¹, 25°C for 15 days. Different culture conditions and medium components (carbon source, nitrogen source, temperature, and pH value) were tested individually for their optimal levels. All experiments were conducted in triplicates and results presented in means values.

2.3 Determination of Optimal Carbon Source

Different carbon sources were tested for the determination of the most optimal carbon source suitable for mycelial growth of the tested strain. The basic medium culture without carbon source was used as a control in this test. Glucose, starch, maltose, sucrose and mannitol each in 20g l⁻¹ concentration were examined for their efficacy in the production of mycelial biomass. The inoculated flasks were incubated in a rotatory shaker (120 rev.min⁻¹) at 25°C and 6.0 pH for 15 days. The carbon source that yielded the maximum mycelial biomass was selected, and its optimal concentration was determined.

2.4 Determination of Optimal Nitrogen Source

Different carbon sources were tested for the determination of the most optimal nitrogen source suitable for mycelial growth of the tested strain. The basic medium culture without a nitrogen source was used as a control in this test. Peptone, yeast, beef extract, ammonia nitrate and urea each in 2g l⁻¹ concentration were examined for their efficacy in the production of mycelial biomass. The inoculated flasks were incubated in a rotatory shaker (120rev.min⁻¹) at 25°C and 6.0 pH for 15 days. The carbon source that yielded the maximum mycelial biomass was

selected, and its optimal concentration was determined.

2.5 Determination of Optimal pH value

Different pH values were tested for the determination of the most optimal pH suitable for mycelial growth of the tested strain. The initial pH values were adjusted to 5.0, 5.5, 6.0, 6.5 and 7.0. 100ml of the basic medium culture was dispensed in each 500ml flask, and each treatment was conducted in triplicate. The flasks containing the seed culture were autoclaved at 121°C for 30 minutes after cooling were inoculated incubated in a rotatory shaker (120 rev. min⁻¹) at 25°C for 15 days. The pH value that yielded the maximum mycelial biomass was determined.

2.6 Determination of Optimal Temperature

Different temperatures were tested for the determination of the most suitable (optimal) temperature for mycelia growth of the tested strain. 100ml of the basic medium culture was dispensed in each 500ml flask at the initial pH value of 6.0. Each conical flask containing the basic medium was inoculated with 4 pieces of 5mm PDA culture and incubated in a rotatory shaker at 15, 20, 25, 30, 35°C for 15 days. Each treatment was examined at different duration of time: 5, 10 and 15 days for determination of the most suitable period for maximum yield of mycelial biomass. The temperature that yielded the maximum mycelial biomass was determined.

2.7 Determination of Optimal Mineral Elements

Different mineral elements were tested for the determination of the most optimal mineral element suitable for mycelial growth of the tested strain. The basic medium culture without mineral elements was used as a control in this test. KH₂PO₄, CaSO₄·2H₂O, MgSO₄·7H₂O, and KH₂PO₄, + MgSO₄·7H₂O, each in 2g l⁻¹ concentration was examined for their efficacy in the production of mycelial biomass. The inoculated flasks were incubated in a rotatory shaker (120 rev.min⁻¹) at 25°C and 6.0 pH for 15 days. The carbon source that yielded the maximum mycelial biomass was selected, and its optimal concentration was determined.

2.8 Analytical Method

After 15 days of submerged cultivation, the culture broth was centrifuged at 5,000 rpm for 20 min. Precipitated mycelia were washed 3 times with distilled water and then dried for 24 h at 60°C. The precipitates were filtered with 0.45 µm Whatman filter paper and then dried in a drying oven to a constant weight. The dry weight of mycelia was quantified by subtracting the dry weight of the filter paper from the total weight.

2.9 Statistical Data Processing and Analysis

All experiments were conducted in triplicates. All data are expressed as the mean (Standard deviation) of three replicates. The data collected were analyzed by ANOVA using statistical tools SPSS 25 version SPSS Statistics 25.0 (SPSS, Inc., Chicago, IL, USA). The means differences were compared by Duncan's test at the 0.05 significance level. Means and standard errors (S.E) from the statistical analysis were brought into Origin Pro 8, and diagrams were created using the line+ symbol and column graph tools.

3. RESULTS AND DISCUSSION

3.1 Determination of Optimal Carbon Source

The carbon source is an essential parameter for stimulating the mycelial growth of Basidiomycetes and Ascomycetes. Carbon source provides the energy needed to promote the growth and development of fungal tissues [29,31]. From Fig. 1, it can be discerned that among the carbon sources studied glucose significantly influenced the mycelial growth of *A. delicata* by attaining the highest mycelial biomass concentration (7.13gl^{-1}), followed by maltose 5.95gl^{-1} , starch 4.61gl^{-1} and sucrose 4.12gl^{-1} relative to the control experiment (Fig. 1). These findings imply that glucose, a monosaccharide sugar, was quickly metabolized to release cellular energy required for mycelial growth as compared to maltose, sucrose (disaccharides), and starch (polysaccharides). Due to its simple structure, glucose can be easily broken down by fungal enzymes. Once glucose is metabolized to release energy for mycelial growth, it accelerates other chemical reactions that produce secondary metabolites. Maltose and sucrose (disaccharides) are the second favourite carbon source; they contain two simple sugar molecules that can be converted into simple

sugar (glucose) and metabolized to release energy and secondary metabolites. Unlike glucose, maltose and sucrose, starch (polysaccharide) was the least metabolized compound due to its complex chemical structure that cannot be readily broken down by fungal enzymes to release energy [31,33]. The results of this study correspond to findings obtained by [32] in the optimization of submerged culture conditions for mycelial growth of *Auricularia auricula-judae* (Agaricomycetes) using glucose as the main carbon source, the mycelial biomass attained was higher as compared to the other carbon sources. Moreover, studies by [33] Optimization of submerged culture conditions for mycelial biomass production by shiitake mushroom (*Lentinus edodes*), the total mycelial biomass recorded using glucose as the main carbon source was 6.73gl^{-1} . Consequently, glucose should be considered as an essential carbon source for the production of mycelial biomass of *A. delicata* due to its immediate energy supply capacity.

3.2 Determination of Optimal Nitrogen Source

Among other factors, the mycelia growth of various macrofungi depends on the availability of nitrogen sources in precise proportion [34-37]. The findings of this study show that yeast was the optimal nitrogen source with the maximum yield of 7.3gl^{-1} mycelial biomass, followed by peptone, which produced 6.8gl^{-1} mycelial biomass. The lowest mycelial biomass was recorded in the medium containing ammonia nitrate 4.7gl^{-1} (Fig. 2). Thus, from these findings, it can be deduced that organic compounds stimulate rapid mycelial growth compared to inorganic compounds. Yeast contains a large content of proteins that are absorbed by mushroom mycelia for growth and metabolite biosynthesis. Yeast extract (an organic nitrogen source), was easily hydrolyzed by fungal enzymes in the culture broth to release nitrogen molecules relative to peptone, beef extract and ammonia nitrate. These molecules undergo biochemical reactions to produce amino acids that constitute mushroom protein. The findings of this study are in line with the studies by [33,37-40], who demonstrated that yeast extract is a significant nitrogen source essential for mycelial growth of *L. edodes*, *T. matsutake*, *P. ostreatus*, *P. cystidiosus*, *G. frondosa*, and *L. decastes* respectively. Compared to inorganic nitrogen sources studied (i.e ammonia nitrate), organic nitrogen sources work best in promoting mycelia

growth relative to inorganic nitrogen sources; thus, yeast extract is essential for increasing the production of mycelial biomass in the culture broth.

3.3 Determination of Optimal pH Value

Initial pH is another essential parameter for mycelial growth of various macrofungi in a submerged culture medium [41-43]. Since the pH of culture medium influences the activities of hydrolytic enzymes, the physiological response of mycelial cell membranes and nutrient absorption, screening its effect on biomass accumulation is of utmost significance [31, 43-45]. Previous studies show that most macrofungi of the genus *Auricularia* produce a large quantity of mycelia biomass at slightly acidic pH values ranging from 5.5 to 6.5. Our current study specified that the ingredients in the culture medium were optimally utilized for mycelia growth at the initial pH of 6.0 (Fig. 3). This implies that at the pH value of 6.0, the mycelial tissues of *A. delicata* were stimulated to release numerous enzymes in the culture broth. Some enzymes catalyzed the breakdown of nutritional compound dissolved in the culture medium while other catalyzes the biosynthesis of secondary metabolites, the formation of cellular structures ultimately promoting mycelial growth [45]. The findings of this study are in accordance with the study by Jo et al., [46] who reported that the mycelia biomass of *A. auricula-judae* was highly accumulated at the pH value of 6.0. Moreover, it was reported that the mycelial biomass of *G. lucidum* and *O. subilludens*, was highly produced at an acidic pH range of 5.5-6.5 [39,47-49]. Determination of optimal pH value is significant for enhancing the mycelial growth of *A. delicata*; thus, 6.0 was the optimal pH required for promoting enzymatic processes that yield high mycelial growth of *A. delicata*.

3.4 Determination of Optimal Temperature

Temperature is an important parameter that influences the growth of fungi. Optimal temperatures stimulate enzymatic activities aiding in the formation of new cells and synthesis of bioactive compounds during mycelial growth [50]. As shown in Fig. 4, the highest mycelial biomass (6.5g l^{-1}) was attained at the temperature of 25°C , followed by 5.4g l^{-1} at the temperature of 30°C . The lowest mycelia biomass (3.1g l^{-1}) was recorded at the temperature of 15°C (Fig. 4). These findings reveal that the optimal

temperature range for mycelial growth of *A. delicata* falls between 25°C and 30°C . This observation suggests that the optimal temperatures activated biochemical reactions involved in cell division and formation of new tissues. The processes of cell division and tissue generation are enzymatically controlled. Since fungal enzymes are functional at specific temperatures, *A. delicata* enzymes attained their maximum activity at the temperature of 25°C - 30°C which resulted to rapid mycelial growth as compared to other temperature values. Moreover, studies on the distribution and habitat of *A. delicata* reveal that it occurs in tropic and subtropics regions with temperature ranging from 25°C - 30°C [51,52]. The habitat information of *A. delicata* concurs with the findings of this study which demonstrate that mycelia of *A. delicata* grow best at the optimal temperatures similar to the temperatures in the tropical regions. The results of this study also correspond to the study by Shah et al. [53] who showed that when the submerged culture media were cultivated at different temperatures, the highest mycelial growth of *G. lucidum* was recorded at 25°C ($276\text{ mg } 100\text{ ml}^{-1}$) and 30°C ($251\text{ mg } 100\text{ ml}^{-1}$). Consequently, optimum temperature plays a significant role in escalating rapid mycelial growth eventually resulting to high production of mycelial biomass of *A. delicata*.

3.5 Determination of Optimal Mineral Elements

Various mineral elements influence vital metabolic processes, biosynthesis and mycelia growth of varieties of macrofungi [54, 55]. Mineral ions enhance catalytic actions of enzymes producing organic molecules such as deoxyribonucleic acids (DNA), ribonucleic acids (RNA), and Adenosine triphosphate (ATP) [56, 57]. These organic molecules play a significant role in promoting the mycelial growth of *A. delicata*. Consequently, during the submerged fermentation process, the determination of optimal mineral elements needed for enhancing the production of mycelia biomass of *A. delicata* is of crucial benefit [21, 58, 59]. Fig. 5, elucidates that the highest mycelia growth was attained in the medium containing the mixture of $\text{K}_2\text{HPO}_4 + \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (6.6g l^{-1}), followed by $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (5.1g l^{-1}), K_2HPO_4 , (4.9g l^{-1}) and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (3.1g l^{-1}) relative to the control medium (Fig. 5). The highest mycelial biomass in the medium containing $\text{K}_2\text{HPO}_4 + \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ is due to the presence of P and Mg minerals. K_2HPO_4 promotes mycelia growth through its

buffering capacity and release of phosphates meanwhile, $MgSO_4$ releases Mg ion, an essential cofactor for some enzymes involved in the biosynthesis of bioactive metabolites [56, 60-62]. The medium containing $CaSO_4$ showed a low mycelia growth due to the capacity of Ca decelerate mycelial growth. These findings collaborate with the discoveries by [62] in the study on the mass production of mycelia of *P. eryngii*, and Vrabl [63] who reported that K_2HPO_4

is an essential bio element for enhancing the biomass production in a batch culture cultivation. In the study done by [60], it was found that K_2HPO_4 and $MgSO_4$ are the optimal mineral elements for maximizing the production of mycelia biomass and polysaccharides of *L. edodes* and *F. betulina*. Consequently, mineral elements (bio-elements) dissociate in a liquid medium releasing mineral ions, of which are a necessary trigger for enzyme activities.

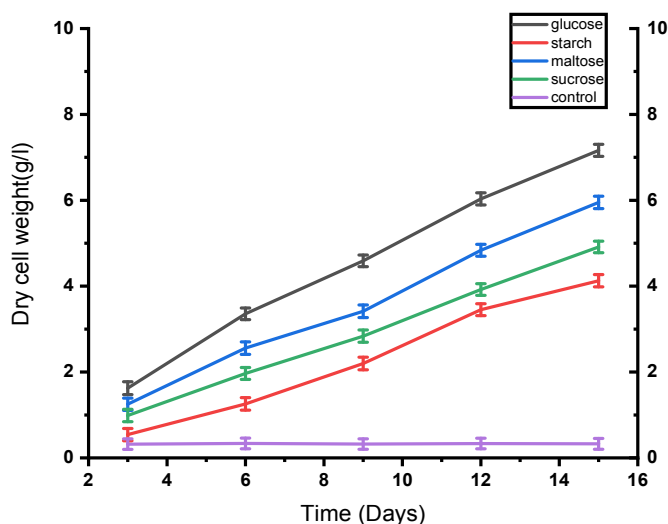


Fig. 1. Effect of carbon source on the production of mycelia biomass of *A. delicata*
 Each data point is the mean \pm S.E. of three replications

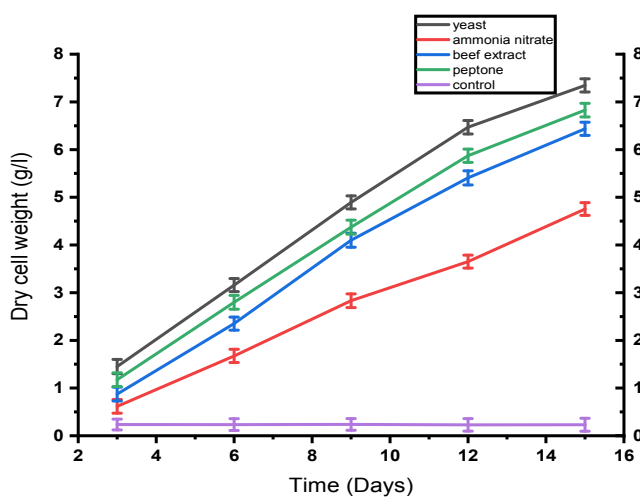


Fig. 2. Effect of nitrogen source on the production of mycelia biomass of *A. delicata*
 Each data point is the mean \pm S.E. of three replications

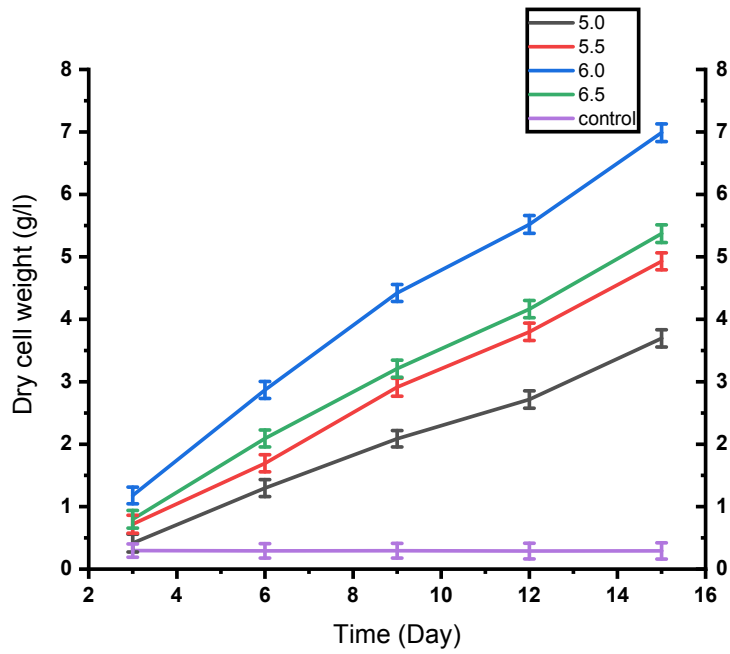


Fig. 3. Effect of initial pH value on the production of mycelia biomass of *A. delicata*
Each data point is the mean \pm S.E. of three replications

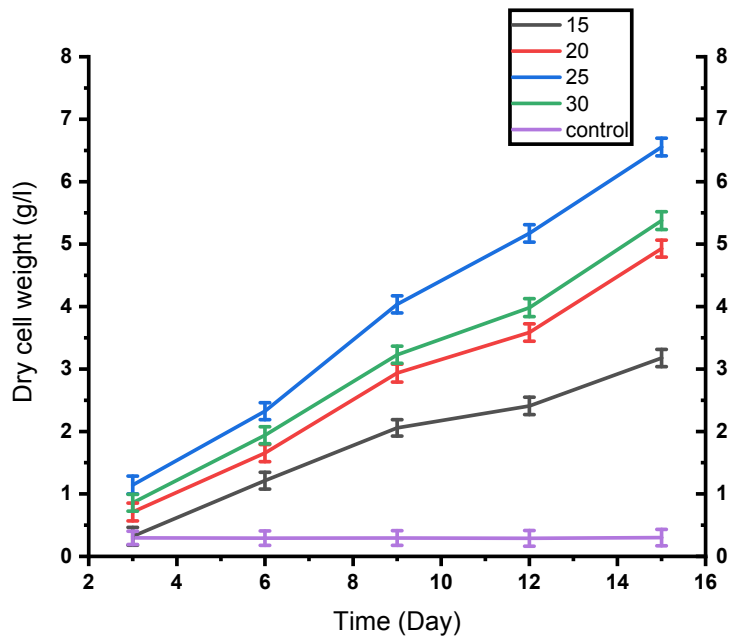


Fig. 4. Effect of incubation temperature on the production of mycelia biomass of *A. delicata*
Each data point is the mean \pm S.E. of three replications

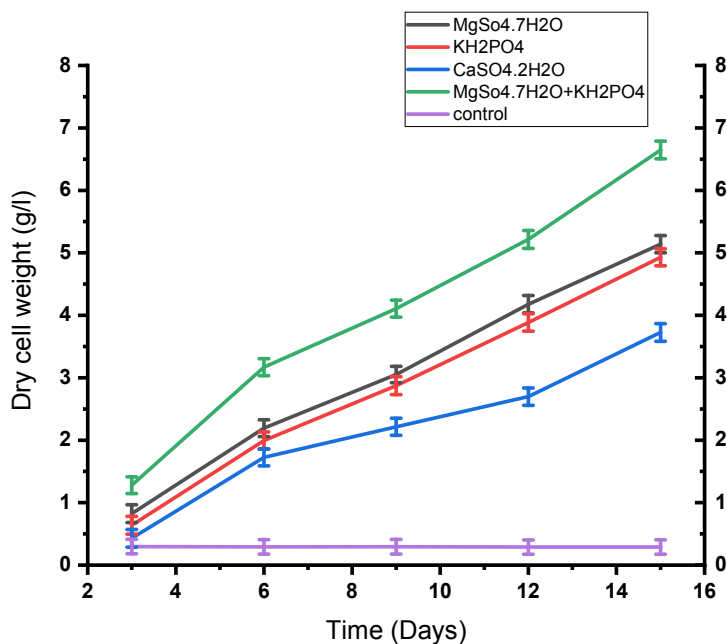


Fig. 5. Effect of mineral elements on the production of mycelia biomass of *A. delicata*

Each data point is the mean \pm S.E. of three replications

4. CONCLUSION

The optimization of culture conditions and medium components is of significant importance for the cultivation of *A. delicata*. Compared to other carbon sources studied, glucose is an optimal carbon source essential for the production of mycelial biomass of *A. delicata* due to its simple structure and immediate energy supply capacity. Organic nitrogen is mostly preferred by fungal mycelia as compared to inorganic nitrogen; hence yeast extract promoted the highest production of mycelial biomass in the culture broth. Determination of optimal pH value is significant for enhancing the mycelial growth of *A. delicata*; thus, 6.0 was the optimal pH required for promoting enzymatic processes that facilitate mycelial growth of *A. delicata*. Moreover, mycelia biomass of *A. delicata* can be efficiently produced at an optimum temperature range of 25°C- 30°C in a submerged culture medium. At optimal temperature, mycelial enzymes catalyze the synthesis of new cells and tissues eventually facilitating rapid mycelial growth. Mineral elements (bio-elements) are essential for enhancing mycelial growth of *A. delicata*; they dissociate in a liquid medium releasing mineral ions, of which are a necessary trigger for enzyme activities. Since submerged fermentation

technology is a cost-effective and time shortening technology, the findings of this study provide a fundamental basis for unveiling the hidden potentials of *A. delicata* including; synthesis of bioactive metabolites, nutritional values, fructification and yield production of *A. delicata* fruit body.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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