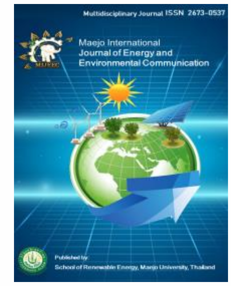
Maejo International Journal of Energy and Environmental Communication

Journal homepage: https://ph02.tci-thaijo.org/index.php/MIJEEC



ARTICLE

Environmental implications and nutrient management: Influence of essential mineral elements on sustainable growth and quality of *Lentinus edodes* in the Qinghai-Tibet plateau

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ARTICLEINFO

Article history:
Received 17 May 2024
Received in revised form
5 June 2024
Accepted 9 June 2024

Keywords:
Mineral elements
Mycelial yield
Agronomic traits
Nutritional composition
Qinghai-Tibet Plateau

ABSTRACT

The study aimed to explore how varying concentrations of calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}) in water influence the growth, yield, and nutritional quality of *Lentinus edodes* (shiitake mushrooms), with the test strain Liao Fu No.4 (0912). *L. edodes* is known for its nutritional and medicinal benefits, making it an important agricultural crop, particularly on the Qinghai-Tibet Plateau, where environmental conditions are unique. The study designed five concentration gradients of these minerals based on their natural occurrence in water from different farms. Results measured the impact of these concentrations on the mycelium growth rate, fruiting body yield, agronomic traits, and the content of polysaccharides and protein in the mushrooms. The results showed that calcium, magnesium, and potassium concentrations significantly influenced both the growth and the nutritional composition of *L. edodes*. It was found that water containing calcium levels between 41-105 mg/L, magnesium between 7.04-8.56 mg/L, and potassium between 7.26-8.85 mg/L promoted faster mycelium growth and higher yields. These optimal concentrations also improved the nutritional quality of the mushrooms by increasing polysaccharide and protein content in the fruiting bodies. Furthermore, the agronomic traits, including size and firmness, were enhanced under these conditions, making the mushrooms more commercially viable. In conclusion, the study provides practical insights for farmers on the Qinghai-Tibet Plateau and similar regions, suggesting that selecting water sources with these specific mineral concentrations can improve both the yield and the nutritional value of *L. edodes*, thereby optimizing mushroom production.

1. Introduction

The Qinghai-Tibet Plateau, often called the "Roof of the World," represents one of the most unique and challenging environments for agriculture due to its high altitude, harsh climate, and limited availability of nutrients. Among the various biological resources adapted to these extreme conditions, *Lentinus edodes* (shiitake mushroom) is a commercially valuable edible fungus with significant nutritional and medicinal properties. However, optimizing the growth and quality of *Lentinus edodes* in such an environment presents distinct challenges, particularly regarding the availability of essential mineral

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elements (Munir et al., 2023a, b).

L. edodes, also known as the shiitake mushroom, belonging to the phylum Fungi, class Basidiomycetes, order Agaricules, family Tricholomatae, and genus Lentinus, is an important cultivated fungus (Sheng et al., 2021). According to the research of Zied & Pardo-Giménez (2017) on the development history of Asian *L. edodes*, China is now the world's largest producer, exporter, and consumer of *L. edodes*. The production of *L. edodes* was initially limited to northeastern Asia but has now expanded to the United States, Canada, Australia, Brazil, and some European countries. Due to its edible and medicinal value, it is expected to be cultivated in more countries shortly. *L. edodes* cultivation has begun to develop in the Qinghai Tibet Plateau; the core focus is to produce high-quality fresh mushrooms in summer. Emphasis is placed on developing off-season edible mushrooms to meet market demand, drive local poverty alleviation, and protect and improve the ecological environment (Yu et al., 2022).

With its fragile ecosystem and unique environmental conditions, the Qinghai-Tibet Plateau poses significant challenges to sustainable agricultural practices, including cultivating *L. edodes* (shiitake mushroom). Given the high altitude, nutrient-poor soils, and harsh climate of this region, managing the input of essential mineral elements such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}) is critical not only for the growth and quality of *L. edodes* but also for minimizing environmental impacts. Excessive or imbalanced use of these nutrients can lead to soil degradation, nutrient leaching, and disruption of soil microbial communities, further exacerbating the vulnerability of the region's ecosystem. Additionally, nutrient runoff risks local water bodies, potentially contaminating freshwater sources and affecting aquatic biodiversity. Therefore, nutrient management in this region requires a careful and sustainable approach (Gruhn et al., 2000; Munir et al., 2021).

One effective strategy involves using organic fertilizers and composts derived from local plant materials, which can improve soil fertility and nutrient retention over time, reducing reliance on synthetic inputs. Precision agriculture techniques, such as soil testing and controlled nutrient delivery, can further optimize the use of Ca^{2+}, Mg^{2+}, and K^{+}, ensuring that the mushrooms receive the necessary nutrients without excess application that could harm the environment. Intercropping *L. edodes* with other crops and implementing soil conservation practices like mulching or cover cropping can also help maintain soil structure, prevent erosion, and improve nutrient cycling. Given the sensitivity of the Qinghai-Tibet Plateau to climate change, these practices need to be climate-resilient, with flexible nutrient management plans that adapt to shifting weather patterns and environmental conditions (Yadav, 2023).

Moreover, integrating traditional knowledge from local communities can enhance these nutrient management strategies. Indigenous practices such as crop rotation and natural soil amendments offer valuable insights into maintaining soil health and ensuring sustainable agricultural practices in this delicate region. By balancing modern scientific approaches with traditional ecological wisdom, cultivating *L. edodes* can become a sustainable agriculture model that supports economic growth and environmental conservation on the Qinghai-Tibet Plateau.

Optimal mushroom growth depends on continuous water supply (Navarro et al., 2020). Dhar (2017) believes that in growing *L. edodes*, water injection from mushroom mycelium is essential. Years of experiments have shown that one mushroom mycelium from the mushroom to the end only needs to be injected with water three times; soaking once in water is the best. With a reasonable arrangement for water injection, the yield of fungus rods will be very high. During the watering process, adding a certain amount of nutrients can effectively compensate for the nutrient limitations caused by mushroom development, prolong the flushes, and increase yields (Klaus et al., 2022). The mushroom's growth period requires an appropriate amount of carbon, nitrogen, mineral elements, and vitamins (Kobayashi et al., 2023).

Previous research on the growth of *L. edodes* focuses on the effects of minerals in cultivation media. However, more research is needed on the impact of mineral content in injection water on *L. edodes* growth during production. Based on the altitude and climate characteristics of the Qinghai Tibet Plateau, this research selected the experimental area with the same altitude and climate, farms with the same cultivation and management mode but a difference of about one-third in yield use of natural water samples for mineral content detection in mushroom cultivation (Table 1). In China, a testing agency, Sichuan Zhonghe Environmental Monitoring Technology Co., Ltd, tested the water sample.

Based on the detection results, Ca^{2+}, Mg^{2+}, and K^{+} with significant differences were selected for experiments, and five concentration gradients were set for Ca^{2+}, Mg^{2+}, and K^{+}according to the differences in detection results; during the experiment, detailed records and data analysis were conducted on the mycelial stage and mushroom emergence stage, as well as the quality testing data of *L. edodes*, to obtain the effects of Ca^{2+}, Mg^{2+}, and K^{+} on the growth and quality of *L. edodes*. At the same time, the Qinghai-Tibet Plateau has a large area, and the water quality and mineral content in different regions vary considerably. Through the use of water with different mineral content in *L. edodes* cultivation, the critical factors affecting the yield of *L. edodes* in the growth process of *L. edodes* were studied. This helps farmers choose areas where planting water is more conducive to *L. edodes* production in site selection. This was to improve the yield and quality of *L. edodes* under the same planting conditions and planting costs and promote the sustainable development of agriculture on the Qinghai-Tibet Plateau.

Table 1 Comparison of natural water sampling and detection data between high-yield farms and low-yield farms.

Index	High-yield farm (mg/L)	Low-yield farm	Concentration Difference(mg/L)
K^{+}	5.67	7.26	1.59
Ca^{2+}	105	169	64
Mg^{2+}	5.52	7.04	1.52

2. Material and methods

2.1 Location of the study

This study was conducted on a mushroom farm in Jiawa Town, Litang County, Ganzi Tibetan Autonomous Prefecture, Sichuan province, China. The location was approximately 2800 kilometers from the capital city of Beijing and 600 kilometers from the provincial capital of Chengdu in Sichuan province. The experimental mushroom farm site was situated at an elevation of 3,600 meters. Longitude 99° 19′ -100° 56′ E, Latitude 28° 57′ -30° 43′ N, Litang 's climate belongs to the plateau climate zone. This was characterized by low temperatures, long winters, abundant sunshine, intense radiation, high wind speeds, synchronous water and heat fluctuations, high evaporation, and distinct wet and dry seasons. The average temperature was 3.0 ℃, with extreme highs at 25.6 ℃ and lows at -30.6 ℃. The average annual ground temperature was 5.9 ℃. The winter was dry, cold, and lengthy, while the warm season was mild and short.

2.2 Test strain

Liao Fu No.4 (0912) strain was obtained from the Liaoning Academy of Agricultural Sciences. Liao Fu No.4 (0912) is a new variety of *L. edodes* jointly researched by the Edible Fungi Research Institute of Fushun Academy of Agricultural Sciences, Liaoning Province, and the Vegetable Research Institute of Liaoning Academy of Agricultural Sciences. Variety L808 is bred through distant hybridization using single-spore hybridization technology.

2.3 Treatments and study design

In the experiment, anhydrous calcium chloride, anhydrous magnesium chloride, and anhydrous potassium chloride were prepared with purified water dissolved into a 5000 mL volumetric flask, preparing experimental water with 5 concentration gradients of Ca^{2+}, Mg^{2+}, and K^{+}. The control group used pure water (Table 2). The study was conducted from January to December 2023, and four replications with 64 bags were used.

Table 2 Experimental water Ca^{2+} , Mg^{2+} ,K^{+} design scheme

number	$CaCl_2$ (mg/L)	MgCl (mg/L)	KCl (mg/L)	control
A	41	4	4.08	Pure water
B	105	5.52	5.67	
C	169	7.04	7.26	
D	233	8.56	8.85	
E	297	10.08	10.44	

2.4 Cultivation substrate

Recipe for *L. edodes* original (cultivated) seed: each bag weighed 1.315 kg, which contained the same amount of 500 grams of sawdust, 60 grams of wheat bran, 5 grams of gypsum, and 0.75 L of water with different concentration gradients as shown in Table 2 and stirred evenly to make mushroom bags and 4 bags for each concentration gradient. Sterilize after making the bags; the high-pressure sterilization pot adopted a pressure of 1.5 Pa, a temperature of 124 degrees Celsius, sterilization for 5 hours, and natural cooling for 24 hours.

2.5. Substrate Inoculation, Cultivation System, and Harvesting

The inoculation method adopted a perforated inoculation method, With holes drilled in the middle of the cultivation bag and the original Strain inserted to ensure the strain fills the entire hole. During the mycelial growth, maintain a room temperature of around 25 ℃. The mushroom rod could enter the color transformation stage when the hyphae formed nodules. After Mycelia thoroughly colonized all the bags, they were moved from the darkroom to a light-filled room for fructification. Maintaining a temperature of 18～22 ℃, ventilate twice a day, once in the morning and once in the evening, each time for an hour. The color change is complete when the stick's surface forms a brown mycelial layer, and white mycelial spots cover less than 10% of the mushroom stick. During this process, the bacterial rod was immersed in water with different Ca^{2+}, Mg^{2+}, and K^{+} concentration gradients for 10 hours to immerse the rod in water fully. After budding, the indoor temperature was controlled within the range of 15-20 ℃, and the relative humidity of the air was around 80~90% (Patil et al., 2024). Harvesting was done when the inner membrane under the mushroom cap began to break. In the Qinghai-Tibet Plateau, the mushroom sticks often reached a dormancy period after only one picking due to the low temperature after the weather entered September.

2.6. Data collection and analysis

Seven parameters were measured throughout the production process from the 64 bags. Including the growth rate of mushroom mycelia (record full bag days), yields (total weight of mushroom), and Agronomic traits (10 fruiting bodies were randomly selected from different mineral concentration treatment groups. Measured the length of the stipe and the diameter and thickness of the cap of *L. edodes*, three times in parallel), Polysaccharide content of fruiting body (phenol-sulfuric acid method), Protein content of fruiting body (Kjeldahl method). Means were separated using the Tukey test when the F-test from ANOVA was significant at $p \leq 0.05$. The results were recorded as mean ±standard deviation and presented in tables.

2.7. Limitations of the study

Due to the limited conditions of the experiment, which was conducted on a farm on the Qinghai Tibet Plateau, it was impossible to collect mycelium for biomass measurement. Only manual tape was used for measurement, and there was a certain degree of subjectivity in collecting mycelium growth status data.

3. Result and Discussion

3.1 The Effect of Ca^{2+}, Mg^{2+}, and K^{+} on the growth of mushroom mycelia

The results showed significant differences in mycelium's average daily growth length under different concentrations of Ca^{2+} treatment. Group A had a superior growth rate to the control group, while the growth rate of other groups was lower than the control group (Figure 1). The Mg^{2+} treatment group showed no significant difference between the A, C, and control groups, while the other groups were all worse than the control group (Figure 1).

In the K^+ treatment group, there was no significant difference between A and the control groups, while the other groups were all worse than the control group (Figure 1).

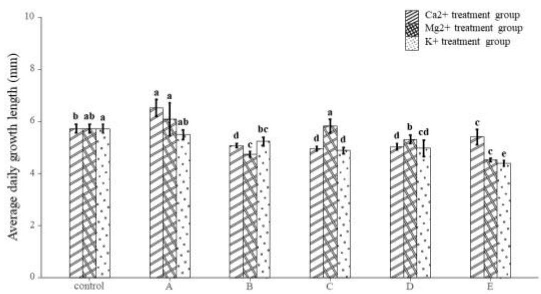

Figure 1 The average daily growth length of mycelium in different Ca^{2+}, Mg^{2+}, and K^+ concentrations

Mineral elements significantly impact the growth rate of the edible fungus mycelium and fruiting bodies' differentiation, development, yield, and quality. This experiment reflects that adding minerals to water injection impacts the growth, quality, and nutritional composition of *L. edodes*. This experiment showed that the mycelium could grow normally under various treatment concentrations, indicating that the selected concentration gradient had no fatal effect on all *L. edodes* mycelia. However, there were differences in the growth rate of the mycelium under different concentrations of Ca^{2+}, Mg^{2+}, and K^+ treatments. The study indicated that a specific concentration of Ca^{2+} can promote *L. edodes* mycelium growth (Tang et al., 2023). Higher concentrations of Mg^{2+} are detrimental to both vegetative and reproductive growth (Wong et al., 2009). Furthermore, it was found that K^+ concentration can inhibit fungal mycelium growth and enhance fruit body primordium formation along with subsequent differentiation and development (Attaran Dowom et al., 2023).

The experimental results showed that with the increase in Ca^{2+} concentration, the growth state of mycelium first increased and then decreased; when Mg^{2+} was added to the injection water, the mycelial growth rate slowed down. Under different K treatment conditions, all treatment groups were lower than the control group. With an increase in K^+ concentration, the growth rate of mycelium gradually decreased.

3.2 The Effect of Ca^{2+}, Mg^{2+}, and K^+ on the yield of *L. edodes*

In the Ca^{2+} and Mg^{2+} treatment groups, there was a significant difference between the control group and treatment groups. The treatment groups were superior to the control group (Figure 2). The results show that as the K^+ increases, the yield rises first and then decreases (Figure 2). During the cultivation of *L. edodes*, especially after fruiting, a lack of nutrients in the substrate can decrease mushroom yield. This can be remedied by adding nutrient solutions rich in nitrogen, phosphorus, potassium, and trace elements to promote mycelial growth and development, thereby increasing production (Kitzberger et al., 2007). From the result, low concentrations of Ca^{2+}, Mg^{2+}, and K^+ could promote the yield of *L. edodes*, while high concentrations of Ca^{2+}, Mg^{2+}, and K^+ have a particular inhibitory effect. According to the experimental results, the yield can be better improved when the concentration of Ca^{2+} in injection water was 41-105 mg/L, the concentration of Mg^{2+} was 5.52-8.56 mg/L, and the concentration of K^+ was 5.67-7.26 mg/L.

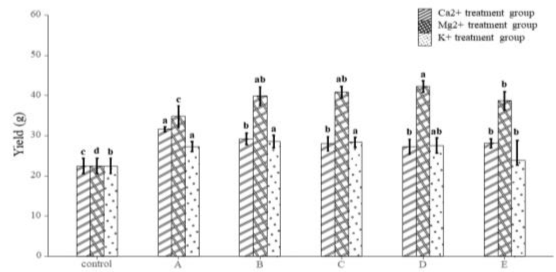

Figure 2 Yields in different Ca^{2+}, Mg^{2+}, and K^+ concentrations

3.3 The Effects of Ca^{2+}, Mg^{2+}, and K^+ on the Agronomic Traits of *L. edodes*

In the Ca^{2+} treatment group, there was a significant difference in the stipe length between the control and other groups, and the data on stipe length was irregular. In terms of cap diameter, there was a significant difference between the control group and other groups, and other groups were better than the control group; the cap diameter increased first and then decreased. Regarding cap thickness data, there was a significant difference between the control and other groups; other groups were better than the control group (Figures 3, 4, and 5). In the Mg^{2+} treatment group, as the concentration of magnesium ions increased, the stipe length first increased and then decreased. Regarding cap diameter, groups A, B, C, and D were better than the control group.

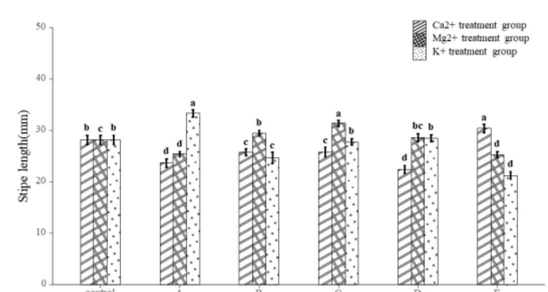

Figure 3 The stipe length in different Ca^{2+}, Mg^{2+}, and K^+ concentrations

Other groups were better than the control group regarding cap thickness (Figures 3, 4, and 5). There was a significant difference between the K^+ treatment groups, but the data did not show a regular pattern of stipe length and cap diameter. Regarding cap thickness, there was no significant difference between groups B, E, and the control group. Adding K^+ to the injection water increased the mushroom cap's thickness (Figures 3, 4, and 5). Adequate supplementation of these minerals in the cultivation substrate can optimize the agronomic traits of *L. edodes*, resulting in higher yields, better quality fruiting bodies, and improved resistance to stress.

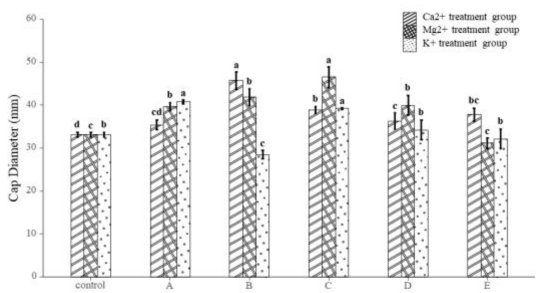

Figure 4. The cap diameter in different Ca^{2+}, Mg^{2+}, and K^+ concentrations

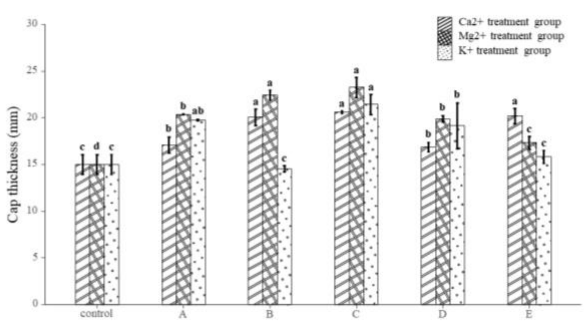

Figure 5. The cap diameter in different Ca^{2+}, Mg^{2+}, and K^+concentrations

There were specific differences in the agronomic traits of *L. edodes* fruiting bodies among different concentrations of Ca^{2+}, Mg^{2+}, and K^+ treatment groups, mainly manifested in the length of the fruiting body stalk, the diameter of the fungus cap and the thickness of the fungus cap. When the Ca^{2+} concentration was 105 mg/L, Mg^{2+} concentration was 7.04 mg/L, and K^+ was 7.26 mg/L, the fruiting body of the *L. edodes* had a short stalk, large-cap diameter, and thicker cap. The study showed that mushrooms with shorter stems, larger caps, and thicker caps were more popular among consumers and had more excellent economic value. (Ning et al., 2021).

3.4 The Effects of Ca^{2+}, Mg^{2+}, and K^+ on the polysaccharide content of *L. edodes*

The results showed a significant difference in polysaccharide content between the Ca^{2+}, Mg^{2+}, and K^+ treatment and control groups, but no pattern existed (Figure 6). In this experiment, different concentrations of Ca^{2+}, Mg^{2+}, and K^+ had no apparent regularity in the content of *L. edodes* polysaccharides. Previous studies have shown that the content of *L. edodes* polysaccharides was associated with harvest time and zinc content in cultivated materials (Ke and Chen, 2016).

In the experiment, after being treated with Ca^{2+}, Mg^{2+}, and K^+, the protein content of *L. edodes* fruiting body increased or decreased compared with the control group, but there was no apparent change. The protein of *L. edodes* was related to the carbon and nitrogen in the cultivated materials (Xiong et al., 2021). This experiment showed that with increased Ca^{2+} concentration in the calcium ion treatment group, all treatment groups' protein content was lower than in the control group. Some studies have shown that adding too much calcium salt to the *L. edodes* culture medium will inhibit protein synthesis and decrease protein content (Jiang et al., 2021).

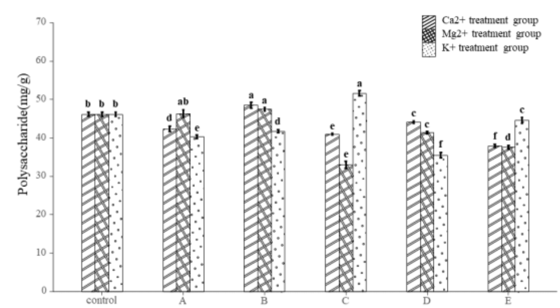

Figure 6. The polysaccharide content in different Ca^{2+}, Mg^{2+}, and K^+

3.5 The Effects of Ca^{2+}, Mg^{2+}, and K^+ on the protein content of the fruiting body

In the Ca^{2+} treatment group, crude protein content was significantly different between the B, C, D, and E groups and the control group, and the crude protein content was lower than in the control group. In the Mg^{2+} and the K^+ treatment groups, there was a significant difference between the treatment groups and the control group, but the data is not regular (Figure 7). The effects of calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^+) on the protein content of the mushroom fruiting body are substantial due to their critical roles in fungal metabolism and physiology. Calcium is essential for maintaining cellular integrity, particularly in stabilizing the cell wall and regulating various enzymatic activities. It influences critical processes such as hyphal growth, spore germination, and fruiting body formation, enhancing protein synthesis within the mushroom. Magnesium is a cofactor for many enzymes involved in energy metabolism and protein synthesis, including those that regulate nucleic acid and amino acid metabolism. This makes magnesium crucial for protein production and overall mushroom biomass. Potassium, on the other hand, is involved in osmoregulation and nutrient transport within the fungal cells. Its role in maintaining cellular turgor and activating enzymes directly impacts mushrooms' growth rate and protein accumulation. Adequate levels of these minerals in the growth medium can promote optimal conditions for protein synthesis, increasing the protein content of the mushroom-fruiting body. However, imbalances or deficiencies in any of these ions may negatively affect the nutritional value of the mushrooms.

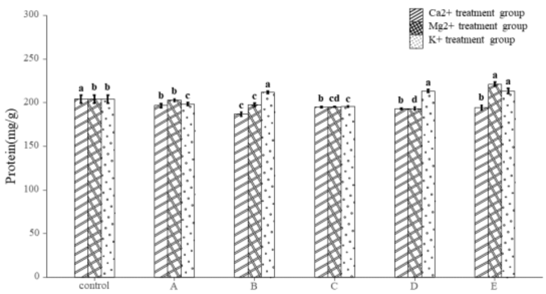

Figure 7. The Protein content in different Ca^{2+}, Mg^{2+}, and K^{+} concentrations

4. Conclusion

The water resources on the Qinghai-Tibet Plateau are rich in minerals. Water sources in different regions significantly impact the yield and quality of Lentinus edodes. Therefore, it was found that injecting Ca^{2+}, Mg^{2+}, and K^{+} water into the growth environment of *L. edodes* positively affects its growth and development. This can help farmers choose more suitable water sources for planting Lentinus edodes, thus saving operation costs and improving yield and quality. According to the experimental results, the yield can be better enhanced when the concentration of Ca^{2+} in injection water was 41-105 mg/L, the concentration of Mg^{2+} was 5.52-8.56 mg/L, and the concentration of K^{+} was 5.67-7.26 mg/L. Compared to the yield of the control group, when the concentration of Ca^{2+} in injection water was 41-105 mg/L can increase the yield by 30～40%, the concentration of Mg^{2+} was 7.04-8.56 mg/L can increase the yield by 82～88%, the concentration of K^{+} was 7.04-8.56 mg/L can increase the yield by 23～27%.

Data Availability

All data supporting this study's findings are included in the paper; however, detailed data can be obtained from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors' Contributions

All authors read and approved the manuscript.

Acknowledgment

The research team thanks the International College of Meizhou University and the Farm in Litang City for providing the experimental environment and related equipment.

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