Cultivation of Low-Potassium *Paddy Straw Mushrooms (Volvariella volvacea)* on Proline-Based Osmolyte Growing Medium to Enhance Nutritional Variety for Hyperkalemia Patients

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Abstract

The increasing variety of food choices has led to a rise in diet-related diseases, as people often prioritize taste over nutritional content. The high consumption of sodium and potassium-rich foods has contributed to a growing number of kidney disease cases. While many studies have focused on producing low-potassium foods, these often result in suboptimal growth. Mushrooms are a nutrient-rich food source, but their high potassium content makes them unsuitable for kidney disease patients. This study aims to determine the effects of different growing media compositions and osmolyte supplementation on potassium content, yield, and mycelium growth duration in low-potassium mushrooms. The cultivation was conducted using the baglog method, starting with the preparation of a growth medium composed of a combination of sengon wood sawdust, rice bran, and dried kepok banana leaves, which were sterilized before inoculating mushroom spawn and incubated in a kumbung for 35 days. Mycelium length was observed during growth, and potassium content was tested post-harvest. The results showed that proline supplementation had an effectiveness threshold, with 3% proline in composition B yielding the best hyphae development and the highest yield of 296.67 grams with the lowest potassium content. For glycerol treatment, 5% glycerol in composition A produced the highest yield and lowest potassium content, while 1% glycerol in composition B resulted in the longest hyphae growth

Keywords: Glycerol; Osmolyte; Potassium; Proline; V. volvacea

1. Introduction

A healthy lifestyle is essential for maintaining overall well-being, especially amid the widespread consumption of sodium and potassium-rich foods. While these minerals are crucial for bodily functions, their excessive intake can lead to health problems, particularly kidney disorders [1]. According to the World Health Organization (WHO), over 800 million people were diagnosed with kidney failure in 2021, with projections indicating a 41.5% increase by 2040. In Indonesia, approximately 700,000 cases of kidney failure were reported in 2018 [2]. Kidney function decline reduces the kidneys' ability to excrete 90% of daily potassium intake, increasing the risk of hyperkalemia [3]. Consequently, kidney failure patients are advised to adhere to a low-potassium diet, limiting intake to 1,500 mg per day, as high-potassium foods can lead to muscle weakness, heart problems, and even death [4]. However, dietary restrictions can also have negative effects, such as constipation and chronic inflammation [5].

Several studies have explored the development of low-potassium foods, such as low-potassium spinach and tomatoes, though these efforts often result in suboptimal growth [6], [7], [8]. Mushrooms, particularly Volvariella volvacea (paddy straw mushrooms), are highly nutritious and considered functional nutraceutical foods [5]. Their ease of cultivation, fast growth, disease resistance, and high market value make paddy straw mushrooms a promising crop [9]. However, their potassium content, ranging from 2,088 to 2,281 mg/g, poses a challenge for kidney disease patients [10]. Therefore, research is needed to reduce the potassium content in mushrooms without compromising their nutritional value or growth.

Successful mushroom cultivation requires a substrate rich in essential nutrients, such as cellulose, hemicellulose, and lignin [11]. Sengon wood sawdust is a common substrate due to its high cellulose content (49.40%), hemicellulose (24.59%), and lignin (26.01%) [12]. However, limited availability of sengon wood necessitates alternative substrates

like dried kepok banana leaves, which contain cellulose (10.85%), lignin (18.21%), and hemicellulose (19.95%) [13]. Additionally, the growing medium should include supplementary nutrients like protein, which can be sourced from rice bran, containing 11.6-13.90% protein [14]. These three materials are selected not only for their nutritional content but also for their relatively low potassium levels: sengon sawdust (0.19%) [15], dried banana leaves (0.05%) [16], and rice bran (0.6%) [17]. Research by Azizah, (2023) has shown that using banana leaves as an alternative substrate can increase protein content by 8.71% [18]. Furthermore, a combination of sengon sawdust, rice bran, and dried banana leaves as a growing medium accelerates the vegetative growth phase of Pleurotus colonies and produces denser growth compared to sengon sawdust and rice bran alone. The strong and porous fiber content of banana leaves also enhances baglog density and moisture retention [19], [20].

Cultivating low-potassium mushrooms involves reducing potassium levels in the growing medium. However, potassium is a crucial nutrient for mushroom growth, and its reduction can inhibit mushroom development. To mitigate the effects of potassium deficiency, osmolytes can be added to the growing medium. Osmolytes act as osmoregulators, providing positive effects on mushroom fruit bodies [21]. The baglog method is widely used in mushroom cultivation because it allows for better control of environmental conditions such as humidity, temperature, and light, while providing a sterile environment that minimizes contamination risks from undesirable microorganisms [22].

In this study, low-potassium Pleurotus ostreatus cultivation was conducted using a combination of sengon wood sawdust, rice bran, and dried kepok banana leaves, with osmolyte supplementation using the baglog method. The data obtained is expected to serve as a reference for cultivating low-potassium Pleurotus ostreatus, providing an alternative food source for kidney disease patients. Additionally, this research offers a potential solution for managing agricultural waste.

2. Method

2.1. Paddy Straw Mushroom Cultivation

The cultivation substrate was prepared in two treatments. The first treatment involved combining the three substrates, sengon wood sawdust, rice bran, and finely ground kepok banana leaves—according to the composition variables: 1:1:1, 2:1:1, 1:2:1, and 1:1:2, without the addition of osmolytes. Each variable had a total weight of 1000 grams, to which water (70%) and sterilized eggshell powder (1.5%) were added. In the second treatment, the same substrate compositions were supplemented with osmolytes: glycerol at 1%, 3%, 5%, and 7%, and proline at 1%, 3%, 5%, and 7%. The 1000-gram mixture was then packed into baglogs, fitted with rings, and sealed tightly. The baglogs were sterilized using an autoclave at 120°C with a pressure of 2 lb for two hours to eliminate contaminants. Each treatment consisted of three baglogs (replicates). In the next step, the sterilized substrate was inoculated with F1 straw mushroom spawn. The inoculated baglogs were incubated in the dark at a temperature of around 25°C and humidity of 60-80% until the mycelium fully developed. After the incubation phase, the baglogs were transferred to a growing room (kumbung) with a temperature of 28°C, humidity of 80-95%, and adequate ventilation to begin the cultivation phase. The mushrooms were harvested when the caps started to open, by pulling the stems out of the baglogs.

2.2. Potassium Content Testing

For potassium testing in the cultivation substrate, consisting of sengon wood sawdust, rice bran, and kepok banana leaves, a 0.5-gram sample was digested with nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 100 mL volumetric flask. The mixture was heated incrementally from 50°C to 170°C, with digestion extended if the solution remained yellow. After digestion, the solution was filtered, diluted, and analyzed using Atomic Absorption Spectrophotometry (AAS) at 766.9 nm. For mushroom harvest testing, a 50-gram sample was dried, ground, and digested similarly. The resulting solution was diluted and analyzed using AAS under the same conditions. Both tests involved multiple repetitions to ensure accuracy.

3. Results and Discussion

3.1. Content in Mushroom Cultivation Substrates

In this study, the cultivation medium comprises three components: sengon wood sawdust, rice bran, and dried kepok banana leaves. Sengon wood sawdust was selected because it is a hardwood that does not contain resin, which

could impede mushroom growth through its extractive compounds. In straw mushroom farming, rice bran is used as a source of carbohydrates, carbon, and nitrogen. It contains nutrients that transform the carbohydrates from rice waste into cellulose, which is essential for providing energy to boost mushroom growth. Furthermore, the carbon in rice bran aids in the development of mycelium and the necessary enzymes [14]. Additionally, dried kepok banana leaves contribute to rapid mycelial growth [18]. For the formulation of the cultivation medium, the following ratios of the components are used (sengon wood sawdust: rice bran: dried kepok banana leaves):

Table 1. Variable composition of growing media.

Variables	Composition Ratio	Substrate Mass (gram)		
		Sengon Wood Sawdust	Rice Bran	Kepok Banana Leaves
A	1:1:1	333.333	333.333	333.333
B	2:1:1	500	250	250
C	1:2:1	250	500	250
D	1:1:2	250	250	500

After conducting a potassium content test on each substrate, the potassium levels in each variable composition per baglog can be determined. The following are the potassium levels for each variable composition per baglog:

Table 2. Potassium levels in variable compositions.

No	Substrate	Kalium Percentage (%)	Variables			
			A	B	C	D
1	Sengon Wood Sawdust	0.25	0.833	1.250	0.625	0.625
2	Rice Bran	0.53	1.767	1.325	2.650	1.325
3	Kepok Banana Leaves	0.31	1.033	0.775	0.775	1.550
Total potassium (gram)			3.633	3.350	4.050	3.500
Total potassium (mg)			3633.3	3350	4050	3500

From Table 2, it can be observed that variations in substrate ratios yield different potassium levels in the growing media for mushrooms (Pleurotus ostreatus). The table shows that composition B, with a ratio of 2 parts sengon wood sawdust, 1 part rice bran, and 1 part dried kepok banana leaves, has the lowest potassium level of 3350 mg. This is due to the high proportion of sengon wood sawdust, which has relatively low potassium content. Conversely, composition C, with a ratio of 1 part sengon wood sawdust, 2 parts rice bran, and 1 part dried kepok banana leaves, has the highest potassium level of 4050 mg. The increase in potassium in composition C is mainly due to the high proportion of rice bran, which is rich in potassium. Composition D, with a ratio of 1 part sengon wood sawdust, 1 part rice bran, and 2 parts dried kepok banana leaves, has a potassium level of 3500 mg. The greater use of dried kepok banana leaves in composition D contributes to a higher potassium level than composition B but lower than composition C. Thus, it can be said that selecting the appropriate substrate ratio is crucial for managing potassium content in the growing media, which can significantly affect the potassium levels absorbed during mushroom growth.

The content of the growing media is a factor in mushroom growth, as stated in Ansoruddin's research (2020) that good mushroom cultivation results come from the growing media used for straw mushroom growth [23]. The better the nutrient content in the straw mushroom growing media, the faster the mushrooms grow. This indicates that what is contained in the growing media will be absorbed by the mushrooms through the mycelium and utilized in mushroom growth [24]. In research focused on cultivating low-potassium mushrooms, potassium levels in the growing media are important to know. A scientific approach is adopted by reducing the potassium levels in the growing media. To achieve this goal, a selection of growing media substrates with naturally low potassium content was made. This substrate selection process is based on the chemical composition analysis, which includes the potassium levels of substrates for mushroom growing media, such as sengon wood sawdust, rice bran, and dried kepok banana leaves. By combining these materials in certain ratios, a growing media with controlled and low potassium levels was successfully created. This approach is based on the principle that mushrooms absorb nutrients, including potassium, from their growing media through a complex metabolic process [25]. By reducing the availability of potassium in the growing media, the aim is to reduce the amount of potassium that can be absorbed by the mycelium and fruiting bodies of oyster

mushrooms. The expectation is that limiting potassium levels in the growing media will result in mushrooms with lower potassium content.

3.2. The Effect of Humidity and Room Temperature on Mushroom Growth

Humidity and temperature are critical for the growth of straw mushrooms, particularly in maintaining optimal conditions within the cultivation room. High humidity and stable temperatures are essential for mycelium development and fruit body formation. Effective management of these factors is key to maximizing yield and ensuring quality. In the study, humidity and temperature were monitored every morning and afternoon to maintain the optimal conditions needed for mushroom growth, resulting in the following data:

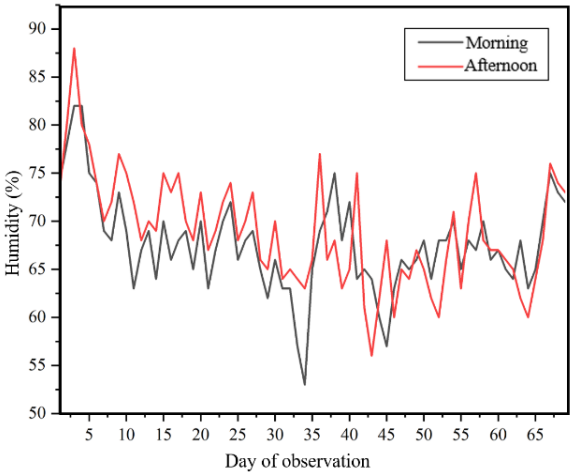

Figure 1. Humidity observations in the cultivation room.

Based on the Figure 1, the humidity data in the mushroom cultivation house shows fluctuations during the incubation and growth process. Data was collected every morning and afternoon before the watering process. Morning humidity peaked on the 3rd and 4th days, April 25th and 26th, with a humidity level of 82%. Conversely, the lowest morning humidity occurred on the 34th day, May 26th, at 53%. For afternoon humidity, the peak occurred on the 3rd day, April 25th, with 88%, while the lowest point was recorded on the 43rd day, June 4th, at 56%. From this data, it can be said that afternoon humidity tends to be higher than morning humidity. This is because during the day, temperatures are generally higher, and sunlight is more intense. This temperature increase can cause evaporation of water from the morning spraying, which ultimately increases the surrounding air humidity in the afternoon.

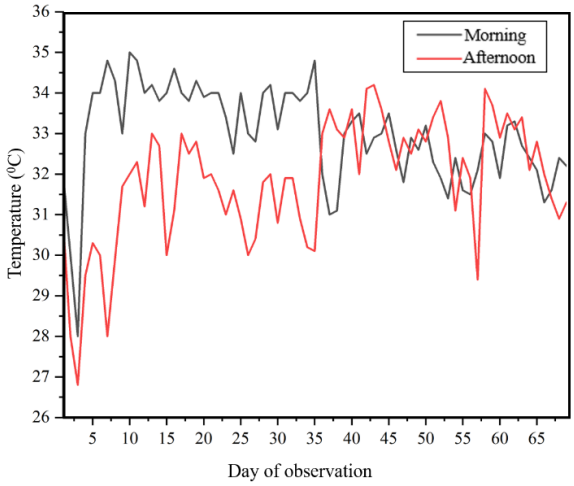

Figure 2. Temperature observations in the cultivation room.

The recorded data shows significant temperature fluctuations in the cultivation room during the incubation and growth phases of the mushrooms, it can be seen in Figure 2. Measurements were taken regularly in the morning and afternoon before watering to monitor environmental changes that could affect the health and development of the mushrooms. Morning temperatures peaked at 35°C on May 10th (Day 18) and dropped to a low of 28°C on April 25th

(Day 3). Afternoon temperatures peaked at 34.2℃ on June 4th (Day 43) and hit a low of 26.8℃ on April 25th (Day 3). These fluctuations reflect the environment's response to external factors such as sunlight intensity, air ventilation, and seasonal changes, all of which significantly impact mushroom growth conditions. During the early mycelium formation stage, mushrooms require high humidity levels of 60-80% for optimal growth, as this supports physiological processes such as water and nutrient absorption. To stimulate pinhead formation and fruit body development, even higher humidity levels of 80-90% are necessary, as the developing mushrooms have greater water needs for intensive metabolic processes. Insufficient humidity below 80% during this phase can hinder nutrient absorption, leading to dehydration, reduced growth rates, and an increased risk of drying out and death.

3.3. Observation of Mushroom Growth

Observations began after the baglogs were inoculated. During the first 7 days, hyphae was observed spreading on the surface of the baglogs. Observations continued for 35 days, during which pinheads were identified on the baglogs in the 4th week. The morphological observations showed that the mushrooms growing were not the expected species, but instead paddy straw mushrooms. In the early growth phase, when the mushrooms were still in the pinhead stage, species identification could not be confirmed. After 2-4 days, the morphological characteristics observed on the mushroom caps indicated a closer resemblance to paddy straw mushrooms (Figure 3).

Figure 3. Paddy straw mushroom.

Contamination in mushroom cultivation involves the intrusion of unwanted organisms such as fungi, bacteria, viruses, or insects into the growing medium or environment. This can occur at any stage of cultivation and can disrupt mushroom growth. Common contaminants include *Trichoderma spp.*, which causes green mold [26]; *Neurospora spp.*, known for orange powdery clumps [27]; and *Mucor spp.*, which results in black pin molds [28], [29]. Figure 4 shows the contaminant morphology These contaminants damage the mycelium and fruit bodies, leading to reduced yields and lower nutritional quality.

(a) (b) (c)

Figure 4. Contamination morphology by (a) *Neurospora sp*; (b) *Trichoderma sp*; (c) *Mucor sp.*

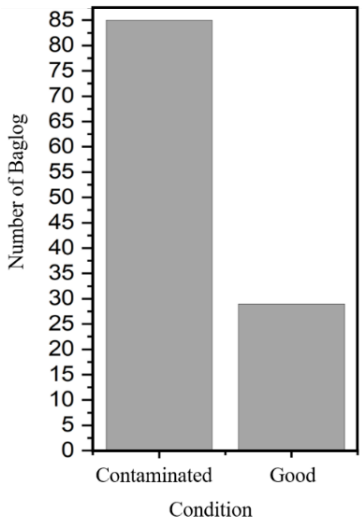

Figure 5. Contaminated baglogs in number.

According to Figure 4, it is evident that the number of contaminated baglogs exceeds the number of baglogs that can develop normally. Out of a total of 114 baglogs produced, 85 experienced contaminations. This issue arises from several factors, including inadequate sterilization, poor cleanliness of the cultivation house, and contaminants introduced by individuals during watering or other activities. These problems are further aggravated by fluctuating humidity and temperature conditions, which create an ideal environment for contaminant growth. Ineffective sterilization allows spores and microorganisms to survive and proliferate, while poor cleanliness and the transport of sterilized baglogs from the laboratory on the 2nd floor to the cultivation house on the 4th floor can increase the risk of pathogen entry. Additionally, contaminants can easily be transferred by people or tools used in the cultivation process. The combination of these factors leads to rapid contaminant growth [30]. The growth of fungal mycelium on contaminated baglogs tends to slow down because the mycelium competes with contaminants for the nutrients available in the growing medium. When contaminants dominate the nutrient sources, the fungal mycelium lacks essential nutrients such as carbohydrates, nitrogen, and minerals. This nutrient deficiency hampers or stagnates mycelial growth, and the mycelium may die due to insufficient nutrients needed for survival. Additionally, some contaminants produce toxic metabolites that damage fungal mycelium. For instance, *Trichoderma spp.* produces enzymes like chitinase and gliotoxin, which damage the cell walls of mycelium. These toxic metabolites disrupt the integrity of mycelial cells, leading to cell lysis and death. As a result, mycelial growth is either halted or significantly reduced [31].

3.4. The Effect of Growing Medium Composition on Mushroom Potassium Content

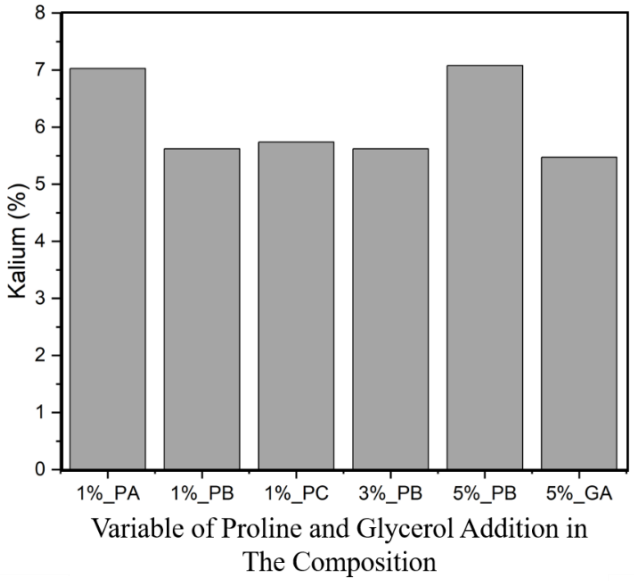

Figure 6. Potassium levels in treatments without contamination.

Figure 6 shows the potassium levels in treatments without contamination. For the 1% proline treatment in composition A, potassium levels were 7.03%, indicating an increase due to proline. In contrast, 1% proline in composition B reduced potassium to 5.82%, and in composition C, it decreased further to 5.74%. Increasing proline concentration to 3% in composition B further reduced potassium to 5.62%. However, at 5% proline concentration, potassium levels rose to 7.08%, suggesting an optimal limit for proline addition. Adding 5% glycerol to composition resulting in a lower potassium level of 5.47%, indicating glycerol's greater effectiveness in reducing potassium compared to proline. Table 3 shows kalium content in mushroom and substrate, it reveals that only about 2 % of Kalium was absorbed in mushroom content. This aligns with Nakakubo et al. (2023), who found that glycerol contributes to increased fruiting body yield and reduced potassium levels by aiding in osmotic pressure regulation Nakakubo dkk., (2023). Data shows that 3% proline reduces potassium levels compared to 1%, but higher concentrations can increase potassium levels due to proline's role in enhancing the uptake of K+, Ca+, P, and N [32].

Table 3. Kalium content in substrate and mushroom.

Sample	Kalium in Substrate (mg)	Kalium in Mushroom (mg)
1%_PA	3633	70.3
1%_PB	3350	58.2
1%_PC	4050	57.4
3%_PB	3350	56.2
5%_PB	3350	70.8
5%_GA	3633	54.7

3.5. The Effect of Growing Medium on Harvest Weight

Table 4. Effect of glycerol addition on harvest weight.

Glycerol	Variables	Fresh weight (gr)	Dry Weight (gr)	Moisture content reduction (%)
1%	GA1	14.139	1.437	90
	GA3	16.901	1.902	89
3%	GA2	47.133	4.946	90
	GA3	44.860	4.625	90
5%	GA1	62.285	6.033	90
	GA2	9.888	1.057	89
	GA3	34.637	3.129	91
1%	GB1	47.138	4.381	91
1%	GC3	19.073	1.768	91
3%	GC1	28.525	3.015	89
	GC2	9.519	1.139	88
	GC3	12.909	1.472	89

The data in Table 3 shows that increasing the percentage of glycerol in the growing medium is associated with a rise in mushroom harvest weight. For instance, in composition A, a 1% glycerol concentration resulted in a harvest weight of 31.04 grams, which increased significantly to 91.9 grams at 3% glycerol and peaked at 106.8 grams with 5% glycerol. A similar trend was observed in composition C, where higher glycerol concentrations also led to greater harvest weights. This effect is largely due to glycerol's role as an osmolyte, which helps maintain cellular osmotic balance, prevents cell dehydration, and ensures that fungal cells remain turgid and nutrient-efficient. This is crucial in potassium-deficient environments, as potassium is key for osmotic pressure regulation and nutrient transport [5]. Additionally, glycerol can be metabolized into glucose, providing essential carbon for energy and cell building [33]. Therefore, higher glycerol concentrations enhance osmotic regulation and energy supply, resulting in optimal growth and increased harvest weights.

Table 5. Effect of proline addition on harvest weight.

Proline	Variables	Fresh weight (gr)	Dry Weight (gr)	Moisture content reduction (%)
1%	PA1	32.682	1.784	95
	PA3	69.437	6.358	91
3%	PA2	47.809	4.640	90
	PA3	4.175	0.452	89
5%	PA1	9.842	0.625	90
	PA3	0.936	0.130	86
7%	PA1	2.047	0.268	87
1%	PB1	107.564	10.729	90
	PB2	60.184	5.743	90
	PB3	11.606	1.186	90
3%	PB1	75.195	7.870	90
	PB2	106.000	12.819	88
	PB3	115.480	14.712	89
5%	PB1	62.920	6.114	90
	PB2	57.571	6.752	88
	PB3	10.484	1.191	89
7%	PB2	4.192	0.988	76
1%	PC1	69.849	6.588	91
3%	PD1	5.202	0.499	90

From the data in Table 4, it can be concluded that a 3% proline concentration in composition B yields the most optimal mushroom harvest. This composition has the lowest potassium level compared to others, indicating that proline plays a crucial role in optimizing mushroom growth in nutrient-deficient media. Research shows that at concentrations higher than 3%, harvest yields tend to decrease, likely due to the negative effects of excess proline accumulation. Proline functions as an osmoprotectant, helping fungal cells withstand osmotic pressure and prevent damage from environmental stress. In nutrient-poor growing media, proline's ability to maintain cell turgidity is vital. Turgidity is the state where cells are adequately hydrated, causing internal pressure to push the plasma membrane against the cell wall, keeping the cells rigid and full. This allows fungal cells to remain metabolically active and absorb nutrients [34]. However, contamination was more prevalent in compositions C and D. Composition C, which has a high rice bran content, also has a high nitrogen content, which can accelerate contaminant growth and hinder mushroom development [35]. The high rice bran content can alter the physical structure of the growing medium, making it either too dense or too loose, which can affect mycelium growth and provide more opportunities for contaminants to thrive [36]. Composition D, with its high content of dried banana leaves, affects substrate moisture. Mushrooms require optimal moisture for growth, and changes in water content can create less-than-ideal conditions, potentially due to the coarse texture of the dried banana leaves.

3.6. The Effect of Osmolyte Addition on Hyphal Growth

Table 6. The effect of glycerol addition on mycelial growth in baglogs.

Glycerol	Variables	Observation (days)					Glycerol	Variables	Observation (Days)				
		7	14	21	28	35			7	14	21	28	35
1%	GA1	4	6.3	8.7	10	11.2	1%	GC1	3.2	4.5	5.2	5	4.8
	GA2	3.2	4.5	5	5.3	6		GC2	2.8	4	4.3	4.2	4
	GA3	4.8	5	8.2	10.5	12		GC3	2.5	4.8	8.2	12.2	14
3%	GA1	3.3	4	4.5	4.8	4.8	3%	GC1	2.5	3.8	8	10.9	15.3

	GA2	4	4.3	8.8	11	12		GC2	3.4	4	7.8	9	11.4
	GA3	2.8	3.6	8	10.7	14.3		GC3	3.2	3	8.2	11	13.6
5%	GA1	3	6.5	10	14.5	16.3	5%	GC1	4.6	9.1	14.5	14	13.6
	GA2	3	7	11.2	13	17.2		GC2	3	4.6	5.5	6.5	6
	GA3	4	8	10.3	12	15		GC3	7	12	13	14.4	14
7%	GA1	4.5	6.5	6	5.5	5.5	7%	GC1	7.5	9.1	11	13	13.3
	GA2	4	6	6.4	6.9	7		GC2	8	9.5	11.5	10.3	11
	GA3	4	5	5.5	7	6		GC3	7.3	9	10	11	11.3
1%	GB1	3.9	7.1	15.5	18.7	20	1%	GD1	6.5	8	10.3	11	10.8
	GB2	4	8.8	14	13.6	14		GD2	4	8	11.6	11	11.2
	GB3	4.5	8	12	10	12		GD3	5.3	8.5	10	10.6	11
3%	GB1	3.5	7.5	11.1	15.3	15	3%	GD1	3.5	8	9	10	11
	GB2	3.4	9	12	13.5	12.7		GD2	4	8.1	8.8	8	8.3
	GB3	3	7	9.2	12	11		GD3	4.2	4	5	6.2	6
5%	GB1	4.2	6.8	9	11	11	5%	GD1	3	5	5.8	6	5.6
	GB2	4	7.2	12	15.5	15		GD2	3	7	7.6	7	7.2
	GB3	4	4.5	5	6.8	7		GD3	3.4	8	9	10	11.2
7%	GB1	4.5	8	11	11.5	11	7%	GD1	4.5	3.6	4	4.2	4
	GB2	4	7.7	10.5	11	11.3		GD2	5	4	4.5	4.3	5
	GB3	3.8	6.8	8.5	8.5	9		GD3	4.7	3	3.8	4	4.3

The Table 5 shows mycelial growth under glycerol treatments observed every 7 days. The results indicate that mycelial length varies with glycerol concentration: composition B with 1% glycerol (GB1) achieved a maximum length of 20 cm, while composition D with 7% glycerol (GD1) had the shortest length at 4 cm due to contamination that halted growth. The medium's composition is crucial for mushroom growth because it provides essential nutrients. Composition B, with the highest ratio of sengon wood sawdust, was most effective in production and biological efficiency [37]. Glycerol's degradation into glucose provides necessary carbon for metabolism [33]. This data highlights that both glycerol concentration and contamination significantly impact mycelial growth, with different glycerol levels producing varying mycelial lengths.

Table 7. The effect of proline addition on mycelial growth in baglogs.

Proline	**Variables**	**Observation (days)**					**Proline**	**Variables**	**Observation (Days)**				
		7	**14**	**21**	**28**	**35**			**7**	**14**	**21**	**28**	**35**
1%	PA1	2.2	4	10.6	15	18.2	1%	PC1	5	7.8	13.6	15.5	18.5
	PA2	3.5	4.4	5	5.2	5		PC2	5.6	8.1	9.6	11.2	12
	PA3	3.3	3.9	4.5	15.2	20		PC3	5	7	7.1	6.6	6.6
3%	PA1	4	4.8	5	5.2	5	3%	PC1	4.8	5	4.5	4.5	4.5
	PA2	5.2	8	12.8	20.5	22.2		PC2	4	4.8	5	4.5	4.5
	PA3	3.6	5	8.4	10.4	15.8		PC3	3.9	4.2	4.9	4.6	4.6
5%	PA1	3	5.2	10	14	18	5%	PC1	5.2	7.7	6	6.2	6.2
	PA2	4.2	7.6	8.8	8.5	8.6		PC2	5	7	5.8	6	6
	PA3	4	5	7.2	10	15.5		PC3	4.8	7.2	5	5.5	5.5
7%	PA1	4	5.8	9.6	12.3	15	7%	PC1	5	7.8	7	6.5	6.5
	PA2	4.5	5	5.2	4.8	4.8		PC2	6.5	9	8.2	7.6	7.6
	PA3	4.2	5	5	4.5	4.5		PC3	5.8	8.8	9	8.4	8.4
1%	PB1	4	8.8	11	18.2	23.6	1%	PD1	5.8	8.5	13	14.2	14
	PB2	3.4	7.9	14	20	23		PD2	4.3	7	11.3	13	13.3
	PB3	3.6	8	11.2	16.5	18.8		PD3	5.5	8.5	14.5	14	15
3%	PB1	4.8	9	17.8	22	22.9	3%	PD1	5.6	7.5	13.7	15	17.7
	PB2	4	9.3	16.6	20.5	23		PD2	5	8	12	11	12.2
	PB3	3.5	8.2	14	18.3	23.8		PD3	6	8	11	12	13

5%	PB1	4	9	15.3	19	22	5%	PD1	4.3	8	13	13.6	14
	PB2	4	6.9	10.3	12.5	13.7		PD2	3.9	6.6	8.8	8.5	8
	PB3	4.5	8	9.1	10	10		PD3	4.8	7	8	8	8.5
7%	PB1	5.7	7	13.9	15.5	15	7%	PD1	3.3	7.2	9	10.3	11
	PB2	4	8.3	10	11	15		PD2	4.2	8	9.2	11	11.4
	PB3	5.2	9	10.3	12	13		PD3	3.1	6	8	10.2	11

The Table 6 shows mycelial growth with proline addition observed every 7 days. Growth varied based on the treatment provided. For instance, in the 3% proline treatment (PB3), mycelial length reached a maximum of 23.8 cm, whereas in the 3% proline treatment (PC2), growth was the shortest due to mycelial shrinkage from contamination. This data indicates that proline addition at the same concentration can result in different mycelial growth, depending on environmental conditions and contamination factors. Proline, as an osmoprotectant, helps organisms maintain osmotic balance under stressful conditions [34], but excessive application can have toxic effects [38].

4. Conclusions

From the research conducted, the following conclusions can be drawn: The lowest potassium content in harvested mushrooms was found with a 3% proline treatment in composition B, at 56.2 mg. Increasing proline above 3% raised potassium levels due to enhanced uptake of K+, Ca+, P, and N. The lowest potassium content with glycerol was 54.7 mg using 5% glycerol in composition A. The optimal harvest weight was achieved with 5% glycerol in composition A and 296.67 grams with 3% proline in composition B. Higher proline concentrations decreased harvest weight, indicating a usage limit. The best mycelial growth was observed b vvvwith 3% proline in composition B (23.8 cm) and 1% glycerol in composition B (20 cm), with proline showing a more favorable impact on growth due to its Osmo protective role.

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