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Potential application of biochar as a growth supplement for mushroom cultivation (*Pleurotus ostreatus*)

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Abstract
The study aims to examine the growth, yield and nutritional values of mushroom (*Pleurotus ostreatus*) to biochar amended substrate from Banmara (*Lantana camara*). The experiment was laid out in a completely randomized design with three replications at Nepal Academy of Science And Technology's Laboratory, Khumaltar Lalitpur. Different concentrations of biochar (2%, 5%, 8%, 10%, and 15%) were utilized as supplements for mushroom cultivation. Results indicate that a substrate with 2% biochar enhances stalk length (7.1 cm) compared to the control (6.8 cm). Mycelia growth was faster with 2% biochar (10 days) versus the control (20 days). Proximate composition shows 23% crude protein in 5% biochar, a 9.35% increase compared to the control (13.65%). Total Biological Efficiency (%) is higher in the control (33%) than 2% and 5% biochar (18.66% and 13.19%). Nitrogen (%) is higher in 5% biochar (4.01) than in control (2.00%). Mineral analysis is significantly higher in biochar-added substrates than in the control.

Keywords: Biochar, concentrations, mushroom, mineral analysis, proximate composition

1. Introduction
Mushrooms are widely celebrated in gourmet cooking worldwide, valued by people for their attractive culinary attributes, particularly their sensory qualities (Patel and Goyal, 2012) [8]. While there exist numerous mushroom varieties (Approximately 2000), the selection of edible mushrooms is restricted to about 25 types, with only a few being cultivated on a significant scale by professionals. Mushrooms, known for their fragility, hold substantial nutritional and functional importance. Recently, they have been recognized for their nutraceutical significance, attributed to their sensory appeal, medicinal properties, and economic value (Chang and Miles, 2008) [3]. Mushrooms serve as a nutritious dietary option, rich in protein, vital amino acids, fiber, potassium, and vitamins (Rafique, 1996) [10]. Unlike green plants, mushrooms do not possess chlorophyll and are unable to undergo photosynthesis. They rely on pre-existing nutrients such as cellulose, glucose, and starch, which they break down using their enzymes to sustain their growth and development (Zadrazil and Kurtzman, 1982) [14]. The oyster mushroom, a member of the Pleurotus species, is an edible mushroom that flourishes in tropical and subtropical areas. It is known for its capacity to thrive on a variety of substrates, including wood, wood shavings, sawdust, and various other materials (Quimio *et al*., 1990) [9].
Biochars are solid materials abundant in carbon, created through the thermochemical decomposition of organic biomasses in an environment devoid of oxygen (Lehmann and Joseph, 2009) [6]. Biochar can be generated from various biomass sources, encompassing animal dung and waste biomass sourced from industrial, forest, horticultural sites, and occasionally agricultural remnants. Due to variations in biomass composition and the conditions of thermal decomposition, biochar lacks uniformity and standardization, making it a non-uniform product (Lehmann and Joseph, 2009) [6].
Biochar is a carbonized organic material employed to improve soil quality, primarily aiming to augment carbon storage. Supporters propose potential agronomic advantages such as enhanced nutrient utilization efficiency, elevated crop yield, and diminished leaching losses,

coupled with immobilization of pollutants. Nonetheless, its efficacy is a subject of controversy, with disparate findings likely stemming from diverse biochar variations and application circumstances, encompassing distinct feedstocks, production parameters, soil types, climates, and crop varieties (Foereid 2015) [4].

Biochar, produced through controlled pyrolysis of organic substances such as wood and plant waste, effectively seizes and preserves carbon in a stable form, resistant to release into the atmosphere. This method not only reduces the emission of pollutants but also creates a source of renewable energy. Agricultural residues, acknowledged for their abundance of carbon, are becoming noteworthy as substantial renewable feedstocks (Bais Moleman *et al*., 2019) [2]. Biochars might exhibit pesticidal properties, holding promise for the development of biopesticides (Sayed *et al*., 2018) [12]. The current focus on biochar revolves around its economic implications. In Australia, the integration of biochar with NPK resulted in a 53% boost in crop yield, leading to a rise in farmer net benefits by $8,000 per hectare, with a biochar cost of $160 per hectare (Robb, Joseph, 2019) [11].

The oyster mushroom (*Pleurotus ostreatus*), in particular, is a popular mushroom that may be farmed in Nepal. It can be grown in the lowlands since it responds to moderate temperatures. Oyster mushrooms may be available for commercialization throughout the year as they have inexpensive manufacturing costs. It is theorized that biochar can function as an enhancer to raise the quality and growth of mushrooms to boost yield productivity and quality. As a result, this research was conducted with the aim to make biochar from the pyrolysis process and using thus formed biochar as a mushroom growth media modification (*Pleurotus ostreatus*). The purpose of this research is to ascertain the efficacy of these substrates in promoting the growth of *Pleurotus ostreatus* and to analyze the nutrient content of biochar in Nepal.

2. Methodology's

2.1 Preparation of Biochar

Biochar was prepared in a steel cone kiln which is the simple traditional way of making biochar and is much more closely associated with the ancient methods of production. The feedstock used for the preparation of biochar was collected from RECAST (Research Centre for Applied Science and Technology, Tribhuwan University, Kritipur, Nepal). Biochar produced from the steel cone kiln in each batch was quenched or snuffed with water. The weight of biochar was measured after water snuffing and after drying. Similarly, the weight of water that was used while snuffing was also quantified. Biochar was prepared using invasive species *Lantana camara*. The processes that were followed in preparing biochar are as follows: First of all, *Lantana camara* was weighted and fed into the end of kiln to heat the feedstock. Pyrolizing biomass layer by layer in a conical-shaped metal kiln. A fire was ignited in the kiln, which was then spread to produce a first layer on the very bottom of the kiln. This was followed by the addition of biomass. The kiln was filled with the feedstock and was allowed to burn properly. After the complete burn of feedstock, quantifying amount of water was poured for absorption. The weighing of the biomass was performed pre pyrolysis and then the biochar formed was weighed later. This enabled us to estimate the percent of biochar produced. Calculation to calculate the biochar yield:

Biochar % = Biochar Mass/ Biomass Mass *100

For the preparation of biochar, the recording was done for each batch. Each batch's biochar percentage was calculated. Three batches of biochar were made in this manner at a temperature of 650 °C. Biochar basic qualities generated from *Lantana camara* were analyzed using samples from the three batches. pH (probe method), carbon (Elemental Analyzer), total nitrogen (Elemental Analyzer), and hydrogen (Elemental Analyzer) were all evaluated, and 0.47% nitrogen, 6.25% hydrogen, and 45.3% carbon were discovered. Biochar was discovered to have a pH of 10.38.

Table 1: Different concentrations of biochar that were used for the experiment

Percentages	Biochar in Kg	Mushroom Substrate in Kg
2%	0.1	4.9
5%	0.25	4.75
8%	0.4	4.6
10%	0.5	4.5
15%	0.75	4.25
Total	2 kilogram	23 kilogram

Biochar percentages of 2, 5, 8, 10, and 15% were computed at first. Each mushroom bag log contained a total of 5 kg of mushroom substrate. In all, 2 kg of biochar was employed for mushroom cultivation with the mushroom substrate. Before being mixed with the sterilized mushroom substrates, each percentage (As stated in Table 1) was correctly labelled and sterilized at 121 ºC for 20 minutes. The weighing equipment was utilized to maintain precise calculations, and the mixture was properly blended and used for the next experiment.

2.2 Source of *Pleurotus ostreatus*

Mush Nepal Pvt. Ltd. Balambu, Kathmandu, Nepal provided the strain of *P. ostreatus* used in this experiment.

2.3 Preparation of mushroom substrate

The oyster mushroom (*Pleurotus ostreatus*) was cultivated on paddy straw substrates, which are extensively employed since straw is readily available and inexpensive. Fresh and well-dried straw was used and provided by Mush Nepal Pvt. Ltd., Balambu, Nepal.

2.3.1 Soaking

Straw was chopped into 3-5 cm pieces and soaked for 8-16 hours in fresh water. The excess water in the straw was drained.

2.3.2 Heat Treatment

Heat treatment of the substrate reduces contamination and leads in greater consistent outputs. Pasteurization is one method. Firstly, Water was cooked in a petroleum drum with a large mouth (Steel). Plastic sacks were used to hold the moist substrate. For around 10-15 minutes, the filled sack was immersed in hot water (80-85 °C). It was crushed with a hefty object (Brick or stone) and a wooden piece to keep it from floating. Excess hot water was emptied from the container after pasteurization so that it could be reused for additional sets.

2.4 Autoclave
Before combining, the different percentages of powdered biochar (2, 5, 8, 10, and 15%) the substrates were sterilized separately. The autoclaved biochar and mushroom substrate were packed in various plastic bag logs, which were then coated with aluminum foil and labeled appropriately.

2.5 Spawn and Spawning
The pasteurized substrate was suitable for filling and spawning once it had cooled to room temperature. The moisture content of the substrate was around 70% at this point. For cultivation, polythene bags (35*50 cm, 150 gauges) were utilized. For 5 kg of wet straw, 200 gm of plastic spawn was used (1 bag). Layer spawning was used for spawning. Substrate was placed in a bag, compacted to a depth of 8-10 cm, and a handful of spawn was sprinkled on top. Similarly, the second and third layers of substrate were placed, and the bags were closed concurrently after spawning. After that, the bags were carefully compacted and sealed for spawn running (Development). The generated bags were piled neatly and cleanly on racks in a closed configuration. Spraying water on the walls and floor twice a day kept the temperature at 18 °C and the humidity at 75-80%.

2.6 Observation
Mushroom bowls were kept inside the room by keeping the temperature at 18 degrees Celsius and the humidity at 75-80%. Watering was done twice a day with the help of spray. The frequent observation was carried out in order to conduct further research.

2.7 Cropping and harvesting
The polythene covers were removed after 22 days, when the bags were thoroughly impregnated with white mycelium. Mushrooms were grown in an 18 °C temperature range.

3. Statistical analysis
All collected data were compiled and entered in Microsoft Excel (2007). Collected data were set in software named Rstat version 1.4.1106, Packages used in R (Agricolae version 1.3-5, readxl version 1.3.1, rstatix version 0.7.0). DMRT (Duncan Multiple Range Test) for mean separation at 5% level of significance was done.

4. Results and Discussion
4.1 Time taken to start mycelia growth
The colonization of the substrate by the mycelia within the bags at five-day intervals was measured to track their progress. After that, the time it took the mycelia to fully colonize the substrate post-spawning was noted (usually in several days). White mycelia formed across the substrates within the bags, indicating colonization.
Mycelial growth showed early development in 2% Biochar added mushroom substrate i.e. in 10 days which is followed by 5% Biochar and control. Meanwhile, no growth was seen in other treatments which are shown below in Table 2.

Table 2: Effect of different percentages of biochar added mushroom substrate in time taken for the growth of mycelia of mushroom (*Pleurotus ostreatus*)

Time interval (Days)/Treatment	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
2%	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes
5%	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes
8%	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10%	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
15%	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
control	No	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes

Note: Yes stands for growth and No stands for non-growth

4.2 Time taken to start *primordia* formation
The creation of *primordia*l was observed and documented every two days after the bags were cut open. *Primordia* formation showed early development in control which is followed by 2% Biochar and 5% Biochar. Meanwhile, no formation was seen in other treatments which are shown below in Table 3.

Table 3: Effect of different percentages of biochar added mushroom substrate in time interval for the *primordia* formation of mushroom (*Pleurotus ostreatus*)

Time interval/ Treatment	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
2%	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes
5%	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes
8%	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10%	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
15%	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Control	No	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes

Note: Yes stands for growth and No stands for not growth

4.3 Length of stalk and perimeter of cap of mushroom
Thirteen fruits were chosen, and the stalk lengths from the tip of the stalk to the base of the caps were measured. This was repeated for each harvest, with the average calculated.
A significant result was seen for the length of the stalk and perimeter of the cap of the mushroom under the different Biochar percent treatments. Among all the treatments 5% Biochar showed a higher length of Stalk which is significantly at par with 2% Biochar which is followed by control as shown in Table 4.

Table 4: Effect of different percentage of biochar added mushroom substrate in length of stalk and perimeter of cap of mushroom (*Pleurotus ostreatus*).

Treatment /perimeter	Length of stalk of mushroom (cm)	Perimeter of cap of mushroom(cm)
2% Biochar	7.7±0.66[a]	33.5±0.40[a]
5% Biochar	8.0±0.55[a]	30±1.20[a]
8% Biochar	0	0
10% Biochar	0	0
15% Biochar	0	0
control	5.8±0.87[b]	30±0.2[b]
mean	3.6	15.67
LSD 0.05	0.89***	0.93***
CV%	13.91	3.365

4.4 Mineral content of mushroom

A significant result was seen for the mineral content under the different Biochar percent treatments. Among all the treatments 5% Biochar showed higher mineral content i.e. sodium, magnesium, potassium, and calcium followed by control and 2% Biochar. 5% Biochar showed a higher amount of nickel, zinc which is followed by 2% Biochar and control. Manganese and iron are seen higher in 5% Biochar which is followed by 2% Biochar and control. 2% Biochar showed a higher amount of chromium which is followed by 5% Biochar which is significantly at par with control which is shown in Table 5 and 6.

Table 5: Effect of different percentages of biochar added mushroom substrate in mineral content of mushroom (*Pleurotus ostreatus*)

Treatment/mineral content	Sodium (g/kg)	Magnesium (g/kg)	Potassium (g/kg)	Calcium (g/kg)
2% Biochar	0.81±0.01[c]	1.33±0.05[c]	35.40±3.0[b]	0.21±0.02[b]
5% Biochar	1.61±0.03[a]	1.83±0.02[a]	39.0±2.1[a]	0.43±0.01[a]
8% Biochar	0	0	0	0
10% Biochar	0	0	0	0
15% Biochar	0	0	0	0
control	1.57±0.01[b]	1.48±0.01[b]	58.04±11.7[b]	0.20±0.005[b]
mean	0.666	0.77	22.07	0.142
LSD 0.05	0.025***	0.0453***	8.91 ***	0.019***
CV%	2.15	3.29	22.70	7.56

Table 6: Effect of different percentages of biochar added mushroom substrate in mineral content of mushroom (*Pleurotus ostreatus*)

Treatment / mineral content	chromium (mg/ kg)	Manganese (mg/kg)	Iron (mg/kg)	Nickel (mg/ kg)	Zinc (mg/kg)
2% Biochar	0.92±0.052[a]	13.33±0.28[c]	62.6±2.35[c]	0.21±0.01[b]	85.10±4.15[b]
5% Biochar	0.60±0.02[b]	50.56±1.00[a]	193.2±1.00[a]	0.83±0.02[a]	112.1±3.8[a]
8% Biochar	0	0	0	0	0
10% Biochar	0	0	0	0	0
15% Biochar	0	0	0	0	0
control	0.60±0.01[b]	18.83±0.05[b]	67.13±1.51[b]	0.17±0.01[c]	76.00±2.0[c]
mean	0.35	13.78	53.83	0.20	45.53
LSD 0.05	0.04***	0.758***	2.15***	0.020***	4.36***
CV%	6.9	3.09	2.25	5.81	5.38

4.5 Nutrients content of mushroom

Significant result was seen for the nutrient content under different Biochar percent treatments. Among all the treatments 5% Biochar showed higher moisture content which is significantly at par with 2% Biochar and followed by control. Likewise, Dry matter showed higher amount in control which is followed by 2% Biochar and 5% Biochar. Total ash, Total fibre, Total carbohydrate, and Crude lipid content showed no significantly difference under different Biochar percent treatments. Total protein content showed a higher amount in 5% Biochar which is followed by 2% Biochar which is significantly at par with control which is in shown Table 7 and 8.

Table 7: Effect of different percentage of biochar added mushroom substrate in nutrients content of mushroom (*Pleurotus ostreatus*)

Treatment/ nutrient content	Moisture content (g/kg)	Dry matter (g/kg)	Total ash (g/kg)
2% Biochar	0.94±0.02[a]	0.06±0.01[b]	0.12±0.03[a]
5% Biochar	0.95±0.01[a]	0.04±0.01[c]	0.11±0.04[a]
8% Biochar	0	0	0
10% Biochar	0	0	0
15% Biochar	0	0	0
control	0.91±0.005[b]	0.07±0.002[a]	0.11±0.04[a]
mean	45.53	0.028	0.057
LSD 0.05	4.36***	0.01***	0.049***
CV%	5.38	20.39	48.56

Table 8: Effect of different percentage of biochar added mushroom substrate in nutrients content of mushroom (*Pleurotus ostreatus*).

Treatment/ nutrient content	Total fibre (g/kg)	Total Protein (g/kg)	Total carbohydrate (g/kg)	Crude Lipid (g/kg)
2% Biochar	0.22±0.04[a]	0.15±0.045[b]	0.533±0.32[a]	0.023±0.015[a]
5% Biochar	0.19±0.01[a]	0.23±0.03[a]	0.45±0.04[a]	0.023±0.015[a]
8% Biochar	0	0	0	0
10% Biochar	0	0	0	0
15% Biochar	0	0	0	0
control	0.20±0.02[a]	0.13±0.04[b]	0.51±0.011[a]	0.024±0.004[a]
mean	0.103	0.086	0.25	0.011
LSD 0.05	0.031***	0.051***	0.24***	0.0159**
CV%	17.22	33.53	53.04	76.1

4.6 Chemical element of Banmara (*Lantana camara*) biomass and processed biochar charcoal

A significant result was seen for the chemical element content in banmara (*Lantana camara*) biomass and processed biochar charcoal. Biochar charcoal contains higher amounts of calcium, potassium, nickel, chromium, iron, zinc, and manganese compared to banmara (*Lantana camara*). Meanwhile, magnesium and sodium content were significantly indifferent.

Table 9: Chemical element of banmara (*Lantana camara*) biomass and processed biochar charcoal

Chemical element	Biochar (g/kg)	Banmara (g/kg)	Mean	LSD 0.05	CV %
Calcium	8.43±0.378[a]	4.23±0.15[b]	6.33	0.654***	4.55
Potassium	15.76±2.79[a]	7.66±2.9[b]	11.7	6.56*	24.7
Magnesium	3.6±1.35[a]	1.5±0.26[a]	2.56	2.20	37.9
sodium	0.05±0.02[a]	0.03±0.003[a]	0.04	0.04	40.4
Nickel	6±1.7[a]	1±0.0[b]	3.5	2.8**	35.4
Chromium	5.2±1.9[a]	1.0±0.0[b]	3.1	3.05*	43.5
Iron	793.8±1.70[a]	107.6±2.87[b]	450.7	5.36***	0.52
Zinc	187.56±2.57[a]	7.60±1.21[b]	97.58	4.56***	2.061
Manganese	107.23±2.4[a]	18.56±2.1[b]	62.9	5.19***	3.64

4.7 Chemical element of mushroom substrate

A significant result was seen for the chemical element content in mushrooms. Zinc was found in the higher amount which is followed by iron, manganese, nickel, calcium, potassium, magnesium and chromium which is shown in Table 10.

Table 10: Chemical element of mushroom substrate

Chemical element	Mushroom substrate (mg/kg)
Calcium	10.87±4.30[e]
Potassium	7.70±2.8 [e]
Magnesium	1.70±0.26[f]
sodium	66.0±4.8[b]
Nickel	21.06±2.7 [d]
Chromium	0.12±0.01[f]
Iron	64.76±1.34[b]
Zinc	76.23±2.21[a]
Manganese	35.36±1.01[c]
mean	31.53
LSD 0.05	4.60***
CV%	8.51

4.8 Yield and Time period of harvest

A significant result was seen for the mineral content under the different Biochar percent treatments. Among all the treatments control showed higher yield which is followed by 2% Biochar and 5% Biochar. Likewise among all treatments less time to harvest was taken by control which is significantly at par with 2% Biochar which is followed by 5% Biochar which is shown below in Table 11.

Table 11: Effect of different percentages of biochar added mushroom substrate in yield of mushroom (*Pleurotus ostreatus*)

Treatment / Perimeter	Yield (g)	Time period of harvest
2% Biochar	350±57.7[b]	40.75±5.12[b]
5% Biochar	237.5±47.87[c]	50.75±8.13[a]
8% Biochar	0	0
10% Biochar	0	0
15% Biochar	0	0
control	625±119.0[a]	35.00±8.16[b]
mean	202.08	21.08
LSD 0.05	85.321***	7.65***
CV%	28.42	24.42

4.9 Element content in mushroom

A significant result was seen for the element content under the different Biochar percent treatments. Among all the treatments Nitrogen content was obtained higher in 5% Biochar followed by 2% Biochar and control. Carbon content was obtained higher in 2% Biochar which is followed by 5% Biochar and control. Hydrogen content was obtained higher in 2% Biochar which is followed by control and 5% Biochar which is shown below in Table 12.

Table 12: Effect of different percentages of Biochar added mushroom substrate in element content in mushroom (*Pleurotus ostreatus*)

Treatment /element	Nitrogen (%)	Carbon (%)	Hydrogen (%)
2% Biochar	2.95	45.73	6.86
5% Biochar	4.01	43.97	6.25
8% Biochar	0	0	0
10% Biochar	0	0	0
15% Biochar	0	0	0
control	2.00	45.66	6.71

5. Discussion

The pH of the biochar-added substrates was increased. This could be due to the biochar supplied as a supplement. The charcoal is naturally alkaline. For healthy mushroom growth and development, the pH of the mushroom substrates should be kept between 5.8 and 6. The elevated moisture content observed in mushrooms with added biochar, as opposed to the control group, can be attributed to the biochar's hydrogen percentage falling within an appropriate range in the mushroom substrates. This suggests that the biochar possesses a considerable porous size, contributing to its exceptional water-holding capability. The advantageous features of biochar, including its potent adsorption and water-retaining capacities, also played a role in this outcome. When compared to the control mushrooms, those supplemented with biochar exhibited the highest nitrogen

percentage (4.01% compared to 2.02%). Additionally, the biochar-added mushrooms showed a decreased quantity of carbohydrates, measuring at 45%. Various minerals like Potassium, Calcium, Zinc, and Iron were significantly higher in mushrooms with added biochar compared to the control. This could be attributed to the porous nature of biochar, which offers a large surface area for absorbing moisture and minerals. Additionally, the increased presence of nutrients is believed to support the growth of mycelium, and the biochar's excellent water-holding ability ensures sufficient moisture during the mushroom's fruiting stage, reducing the risk of young mushroom production being affected by water scarcity. Furthermore, the characterization data showed increased amounts of potassium, iron, and zinc in the biochar, suggesting that the mycelia absorbed nutrients from the substrates, promoting their overall growth and development.

In other studies as well, when biochar was used along with the recommended fertilizer amount, it resulted in more stems, tubers, and increased weight of tubers per hill. This, in turn, led to an overall higher yield of potatoes (Ali, 2017) [1]. Biochar raised mushroom production by holding onto substrate moisture and decreasing the loss of nutrients (Hu, 2022) [5]. Using biochar along with synthetic fertilizers significantly boosted potato growth, resulted in higher tuber yield, and improved the overall quality of potatoes (Mollick *et al*., 2020) [7].

6. Conclusion

Pleurotus ostreatus, also known as "Kanya chyau" in Nepali, is the greatest mushroom species for eating, according to our studies. Commercial mushroom growing is a profitable venture. The length of the stalk and the perimeter of the mushroom cap were found to be larger in biochar-supplemented mushrooms than in control mushrooms. There was a considerable increase in protein when proximate analysis data from dry matter was compared to control. Different chemical elements, such as potassium (K), zinc (Zn), and iron (Fe), were shown to have a wider range than the control. Mineral analysis of Sodium, Magnesium, Potassium, and Calcium was higher in the biochar-added substrates when compared to the control. The usage of biochar can be expanded for the sake of human health and other advantages, such as waste utilization. The nitrogen percent was also increased by 2.00 percent as a result of the elemental analyzer results. This suggests that if an experiment is continued with the addition of nitrogen to the biochar in any way, the protein increment will be considerable. Due to time constraints, none of the work could be completed in Nitrogen. If the experiment is carried out further, there will be a lot of important work that can be done. As a result, by increasing the protein percentage in mushrooms using biochar, we can improve people's health while also saving money for the country. Instead of using protein capsules, people might take mushroom with biochar as a supplement.

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