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Effects of substrate formulation on yield and yield components in enoki (*Flammulina velutipes*) mushroom cultivation

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Abstract

Mushroom cultivation is considered a technology that transforms waste into valuable food and it is an important technology for sustainable agriculture and waste management. This study was conducted to evaluate the cultivation potential of *Flammulina velutipes* (enoki) mushroom on different agricultural waste-based substrates. Nine different substrate formulations were prepared using locally available wastes such as cotton gin waste, poplar sawdust, wheat bran, sodium carboxymethyl cellulose and compost (vegetable-fruit waste). Barley spawn of enoki (EN141) was used for cultivation. The physicochemical properties of the substrates (moisture, pH, organic matter, C/N ratio) and fungal growth parameters (mycelial growth duration, primordium formation, yield, biological efficiency-BE, protein and mineral content) were investigated. According to the results, the substrates containing 100% cotton gin waste and 80% cotton gin waste + 20% wheat bran provided the highest yield (197.05 g/kg and 185.74 g/kg, respectively) and BE (69.27% and 58.20%, respectively). However, protein content was relatively low in these substrates (27.58%and 21.51, respectively). In contrast, the substrate containing 80% sawdust+15% wheat bran+ 5% cellulose offered the highest protein content (52.85%), but was disadvantageous in terms of yield and fruiting body weight. Compost-based substrates did not support mycelial growth. Mushrooms grown on sawdust-based substrates had high levels of phosphorus, potassium and microelements. The study revealed that substrate selection significantly influenced yield, quality and growth duration in enoki cultivation. Local sources such as cotton gin waste were found to be suitable for high yields, but substrate optimization was needed to increase protein and mineral content. These findings have important implications for sustainable mushroom cultivation and utilization of agricultural wastes.

Key words
Substrate optimization, enoki, biological efficiency, agricultural waste, protein

Introduction

Fungi are organisms with approximately 1.5 million species, of which only 69 thousand are recorded in the literature (Listiana et al., 2024). About 2 thousand of these species are edible, but only 200 are commercially used (Bains et al., 2021). Mushrooms have an important place in the food sector with their high protein, carbohydrate, vitamin and mineral content, in the health field with their medicinal properties and in biotechnological applications (Azeem et al., 2020). The most common species in commercial production in Europa are *Agaricus bisporus* and *Pleurotus ostreatus*, and production of species such as Lentinula edodes, *Flammulina velutipes* (enoki) and *Volvariella volvacea* has increased over time (Gupta et al., 2018). These five genera account for 85% of world mushroom production. Enoki species ranks 5th with 5% production (Royse, 2014).

World mushroom production increased to 42.792.893 tons in 2020 (FAO, 2021). Approximately 72.000 tons of mushrooms are produced in Turkey (TUIK, 2023). China is the leading producer of enoki mushrooms with an annual production of 2.4 million tons (Liu et al., 2018). Enoki production has not yet been commercialized in Türkiye.

Substrate composition and additives are the most critical factors affecting the growth, productivity, quality and

biological efficiency (BE) of mushroom. For species such as enoki, lignocellulosic residues (cellulose, hemicellulose, lignin) are the main food source and it is important to enrich these substrates appropriately. Substrates with high nitrogen (N) content increase mycelial growth rate. Additional materials such as wheat bran and sugarcane bagasse usually provide this content. Lignocellulosic materials and N sources directly affect growth (Harit et al 2014; Hiramori et al 2017,). Substrates including coffee waste, corncobs, cottonseed hulls and fermented materials provided high BE (Song et al., 1993; Ji et al. 2001; Jung et al., 2009; Khereba et al., 2010; Miao et al. 2014; Kurat and Koh 2017; Han et al., 2024). Substrates consisting of materials such as rice straw and sawdust used alone have been reported to result in low yields (Song et al., 1993; Tang et al., 2001; Khereba et al., 2010; Harit et al., 2014; Miao et al., 2014). The nutrient content of enoki grown on substrats containing wheat bran or soybean meal was found to be rich in protein and amino acids (Han et al., 2024).

Mushroom cultivation is considered a technology that transforms waste into valuable food. Mushroom cultivation is an important technology for sustainable agriculture and waste management. The use of agricultural waste in mushroom substrats has become a sector that creates employment while reducing environmental pollution. The nutritional and medicinal values of mushrooms increase their consumption.

Enoki is one of the special mushrooms that can grow wild in many regions of the world (Hughes et al., 1999; Ge et al., 2015; Rezaeian and Pourianfar, 2017). Enoki, known as winter mushroom, is a type of mushroom that can grow at low temperatures and has high nutritional value. It stands out with its rich protein, carbohydrate, fiber and mineral content.

Enoki cultivation can be economically advantageous by utilizing agricultural waste and using low-cost substrates. However, it is necessary to optimize substrate formulations and C/N ratio to suit local conditions. Improving production techniques and reducing costs, increases the importance of research in this field (Imtiaj and Rahman, 2008; Barmon et al., 2012).

To reduce production costs and for economical mushroom cultivation, it is crucial to formulate a substrate that is locally available and has a high impact on biological productivity and quality. The use of locally continuous and readily available agricultural wastes would also be beneficial for bioconversion. Therefore, more research is needed to identify efficient substrate formulations.

The aims of this study were to use agricultural wastes in enoki substrates and to determine their effects on yield and quality in order to commercialize enoki mushroom, improve production techniques and reduce cultivation costs.

Material and Method

This research was conducted in Eskişehir Osmangazi University, Faculty of Agriculture, Department of Horticulture. *Flammunina velutipes* strain EN141 (Chinese-Korean hybrid) was used. Spawn wrapped in barley were obtained from Ataturk Horticultural Cultures Central Research Institute (Yalova, Türkiye).

Cotton gin waste, poplar sawdust, wheat bran, compost and cellulose (Sodium Carboxymethyl Cellulose-CMC) were used as substrate materials. Compost was obtained by rapid composting method using GEC (Green Enterprise & Co, Malaysia) brand BMC-50 model composter. With sufficient humidity, air and temperature, the aerobic fermentation process of organic wastes was completed by means of highly thermophilic microorganisms. Compost was produced with this machine within 24 hours in Eskişehir, Espark Shopping Mall. For the compost, 50% vegetable-fruit wastes and 50% sawdust were used.

Nine substrates formulatins were prepared in the experiment (Table 1). The homogenously mixed and moistened materials were weighed and filled into heat-resistant polypropene bags at a rate of 1 kg in the proportions specified in Table 1. Plastic sticks were placed in the center of the bags to provide the necessary space for mycelial inoculation. The bags were closed with filtered plastic caps and kept in a pressureless steam sterilizer at 100°C for 5 hours and the substrates were sterilized.

Table 1. Substrats formulations

Substrate Number	Substrate formulation
S-1	100% Cotton gin waste
S-2	80% Cotton gin waste + 20% Wheat bran
S-3	80% Cotton gin waste+ 15% Wheat bran + 5% CMC
S-4	100% Poplar sawdust
S-5	80% Poplar sawdust + 20% Wheat bran
S-6	80% Poplar sawdust + 15% Wheat bran + 5% CMC
S-7	100% Compost
S-8	80% Compost + 20% Wheat bran
S-9	80% Compost + 15% Wheat bran + 5% CMC

Mycelial Inoculation, Incubation and Production of Mushroom

The caps of the bags were opened and the sticks were removed for spawn inoculation on the substrates that came to room temperature. Approximately 5-7 g of barley spawn were added to each bag. The caps of the inoculated bags were closed again and taken into the incubation room. The bags were kept in the incubation room at 24±2°C, 80-85% humidity, and in a dark environment for mycelial development. The bags that completed mycelial development were taken to the production room and the caps of bags were opened. Room humidity was kept at 80-90% and temperature at 13-15°C. Fruiting bodies with stems of approximately 14-16 cm were harvested.

N% content of the substrate samples was determined according to the Kjeldahl method (Kacar and Inal, 2008). 1 g of the dry mushroom sample was weighed. It was burned in the ash oven at 525 ± 25 °C until the ash color turned to white. Ash content was calculated as percentages. Organic matter (%) was calculated by subtracting the ash value from 100 (Kacar and Inal, 2008). Carbon (C) was calculated as 50% of organic matter (Cormican and Staunton, 1991). C:N ratio was calculated from the determined N and C values. The mineral matter of fruitting body measured by ICP-OES (Kacar and Inal, 2008).

Mycelial growth, primordium and fruit body formation duration were determined in days. The amount of fruiting body collected was evaluated as yield per bag (g bag^{-1}). BE% was determined by dividing the fresh mushroom yield by the dry weight of the substrate (Bernabe-Gonzalez et al., 2015).

Mushroom weight (g), cap diameter (mm), stem length (cm) and protein (%) content were determined. Protein content was determined by multiplying the N content of the mushroom samples by a coefficient of 6.25.

Results and Discussion

Various properties of different substrate formulations are presented in Table 2. The moisture content of the substrates varied between 63.15% and 71.58%. The highest moisture was found in S-1 and the lowest in substrate S-7. Researchers reported that the moisture contents of substrates prepared for enoki cultivation were among 49.43-67.29% (Okuyucu, 2021), 69.19-78.89% (Karasoy, 2022), 59.25-63.68% (Baybas, 2023). All formulations were slightly acidic to neutral (pH 6.30-7.93). The highest pH value was measured in S-7 and the lowest in S-9. In general, due to its direct effect on fungal nutrient metabolism, the pH of the substrate affects a wide range of growth characteristics such as BE, yield, and primordium duration (Dowom et al., 2019). It has been shown that pH values between S-4 and S-8 favor mycelial growth in many fungal species, including *Flammulina species* (Osman et al. 2014; Hassan et al. 2012).

The optimal pH for mycelial growth of *Flammulina species* is 5.0-6.5 (Kozhemyakina et al. 2010; Fidler et al. 2015, Reader, 2021), while a relatively neutral pH 6-7 (Harith et al. 2014; Khan and Chandra 2017) is desired for fruiting body formation.

Table 2. Moisture (%), pH, organic matter (%), ash (%), C (%) determined in the substrates, N (%) and C/N values

Substrate	Moisture %	pH	Organic Matter %	Ash %	C %	N %	C/N
S-1	71.58	6.86	96.49	3.51	48.24	0.80	60.30
S-2	67.89	6.56	95.47	4.53	47.73	0.78	61.19
S-3	68.30	6.72	95.26	4.74	47.63	0.82	58.08
S-4	68.42	7.92	97.12	2.88	48.56	0.78	62.31
S-5	67.04	7.48	97.27	2.73	48.63	0.75	64.84
S-6	67.58	7.37	95.41	4.59	47.70	0.60	79.50
S-7	63.15	7.93	93.84	6.16	46.92	3.27	14.34
S-8	63.70	6.34	94.93	5.07	47.46	1.67	28.34
S-9	66.03	6.30	94.33	5.67	47.16	1.12	42.11

The organic matter content in the substrate varied between 93.84% and 97.27%. The highest organic matter was determined in S-5 and the lowest in S-7. Similarly to Baybas (2023) reported that the organic matter content of the substrate using sawdust was 96.67%.

The ash content, which refers to the inorganic residue in the substrate, is inversely proportional to the organic matter. The values varied between 2.73% and 6.16%. The highest ash content was observed in S-7 and the lowest in S-5. Okuyucu (2021) determined the ash content between 7.72-11.81% in different substrate formulations used for enoki cultivation and Baybas (2023) determined between 3.33-9.08% depending on the substrate.

Since C constitutes half of the organic matter, the values are in parallel with the organic matter. C varied between 46.92% and 48.63%. The values obtained were similar with different substrate compositions. Researchers have determined C values of 44.09-46.14% (Okuyucu, 2021), 47.86-48.99% (Karasoy, 2022), 45.46-48.34% (Baybas, 2023) in enoki substrates. The N content in the substrate was significantly higher in S-7 (3.27%) and S-8 (1.67%) than the others. S-7, S-8 and S-9 were mainly composed of compost. Therefore, they contain high N compared to

the other substrates. In substrates other than these value is between 0.60 and 0.82%. Rezaeian and Pourianfar (2017) reported 0.81-1.68%, Sangkaew and Koh (2017) 0.3-3.3%, Okuyucu (2020) 0.55-1.27%, Karasoy (2022) 0.17-1.87% and Baybas (2023) 0.89-3.5% N ratio in enoki mushroom growing medium. The C/N ratio, which gives information about the rate of decomposition of organic matter in the substrate, was low in S-7 (14.34) and S-8 (28.34). This indicates faster decomposition. In other substrates, this ratio varies between 58.08 and 79.50. S-1 had the highest moisture and organic matter content and low C/N ratio. S-7 and 8 were characterized by high N content and low C/N ratio. The C/N ratio ranges of 27.25-62.16% (Rezaeian and Pourianfar 2017), 24.00-33.00% (Kurata and Koh 2017), 35.20-80.70% (Okuyucu 2021), 13.81-58.54% (Baybas 2023) determined in previous studies were found to be compatible with the values we obtained in the experiment.

Table 3 contains data on mycelial development, primordium duration and time to harvest on different substrates. The differences among the substrates were statistically significant.

The shortest mycelial development duration was similarly determined in S-2 (24.66 days) and S-3 (25.66 days). Again, similarly, S- 4 (32.00 days), S-5 (32.33 days), S-6 (31.67 days) and S-8 (31.66 days) completed mycelial development in longer times. Mycelial development was slower in these substrates. Mycelial development did not occur in S-7 and S-9. Depending on the substrat contents, mycelial development duration can vary between 20-59 days. Harith et al. (2014) reported the mycelial development duration as 31-50 days in 22 different substrate. Hiramori et al. (2017) reported it as 24-25 days in the substrate using apple pomace, and Liao et al. (2019) found it to be 20-25 days when rapeseed straw was used, Okuyucu (2021) found it to be 32.8-59.3 days in different growth environments and Baybas (2023) found it to be 24.64-41.53 days in substrates using pomace. In a short time, S-1, S-2, S-3 and S-8 formed primordium in similar times (31.33-33.33 days). The average time for these substrates was in the same statistical group. On the other hand, S-4, S-5 and S-6 formed primordium in a longer time (43.66-45.33 days). In different studies, the primordium period of enoki has changed significantly according to various cultivation conditions. Hiramori et al. (2017) found it to be 46-57 days, Liao et al. (2019) reported it as 35-46 days, Baybaş (2023) as 14.52-19.73 days and Han et al. (2024) as 42.26-44.75 days.

Table 3. Mycelial growth, primordium and fungus formation duration according to substrate formulations

Substratate	Mycelial growth day	Primordium duration day	Fruiting body harvest day
S-1	26.00a	33.00b	61.00cd
S-2	24.66a	31.33b	68.66b
S-3	25.66a	33.33b	64.00c
S-4	32.00b	45.33a	56.33e
S-5	32.33b	43.66a	72.66a
S-6	31.67b	44.00a	69.00b
S-7	-*	-	-
S-8	31.66b	33.33b	58.66de
S-9	-	-	-
LCD P < 0.01).	3.603	3.189	3.442

*No data. Mean values marked with a different letter in each column are statistically different from each other

S-4, which has slow mycelial development, has the fastest harvest time of 56.33 days. This shows that a fast production process occurs after the mycelial development is completed. S-4 seems to be the most suitable for fast harvest. The harvest time was determined as the latest in S-5 (72.66 days) and S-2 (68.66 days). Mycelial development was fast in S-2 and S-3, but the harvest time was moderate. It was determined that S-5, which was found to have both long mycelial development and harvest time, may be disadvantageous in terms of productivity. Data obtained from different studies on the cultivation periods of enoki report that the period from spawn inoculation to harvest varies between 31-77 days depending on the substrate type and growing conditions. Khereba et al. (2010) reported the cultivation period as 59-61 days, Kurata and Koh (2017) as 57.2-76.8 days, Sangkaew and Koh (2017) as 57.0-65.44 days, Hiramori et al. (2017) as 50 days, Liao et al. (2019) as 35-46 days when rapeseed straw was used and Baybas (2023) as 31.61-35.92 days when pirina substrate was used. Especially, alternative substrates such as pomace and rapeseed straw provided shorter cultivation periods compared to traditional methods.

Table 4 shows the values of various properties and statistical analysis results according to different substrate formulations. Substrates were examined in terms of yield, BE, mushroom weight, cap diameter, stem length, protein content and it was determined that the differences in all the mentioned parameters were significant ($p>0.01$).

Mushroom yields varied between 21.58-197.05 gkg^{-1}. The highest yields obtained were measured as 197.05 $gkg^{-1}$and 185.74 gkg^{-1} from S-1 (100% cotton gin waste) and S-2 (80% cotton gin waste + 20% bran), respectively. The lowest yield was obtained from S- 4 (100% Sawdust), while no yield was obtained from S-7 and S-9. When the BE ratio was evaluated, the highest BE was determined in S-1 (69.27%), where the yield per bag was high. This was followed by S-2, S-3 and S-8, which were statistically no different between them. The lowest BE was

measured in S-4 (6.84%). The heaviest mushrooms were measured in S-2 (3.67 g). The mushroom with the largest cap diameter was measured in S- 3 (30.88 mm), and the mushroom with the longest stem length was measured in S-7 (14.74 cm). The yield and BE obtained by different researchers using various substrates in enoki cultivation exhibit significant differences. Various studies have been reported yields and BE respectively for different substrates: Leifa et al. (2001) 45.8 g bottle^{-1}, BE 56-78% in coffee husk and ground coffee waste, Khereba et al. (2010) 212.6 g bag^{-1}, BE 64.53% in corn cob, Harith et al. (2014) 77.63-85.93 g bag^{-1}, BE 150.89-185.09% in rice straw and palm waste, 233.1 g bag^{-1}, BE 132.8% in fermented corn stalks, Xie et al. (2017) 159.4-359.1 g bag^{-1}, BE 53.1-119.7% in ramie stalk, Liao et al. (2019) reported the best result in rapeseed straw as 50.9-100.9 g bottle^{-1}, BE 39.3-80.3%, Okuyucu (2021) in wheat straw+bran as 219.79 g/kg, BE 61.31%, Karasoy (2022) in 12 substrates as 89.60 g bottle^{-1}, BE 40.03% and Baybas (2023) in pomace as 127.72 g bag^{-1} g kg^{-1}, BE 19.29-27.37% (FV008 isolate), 198.12 g kg^{-1}, BE 48.12% (FV010 isolate).

Table 4. Bag yield (g/kg), BE (%), mushroom weight (g), cap diameter (mm), stem length (cm), protein ratio (%) values according to substrate formulations

Substrate	Fruiting body yield gkg^{-1}	BE %	Mushroom weight g	Cap diameter mm	Stem length cm	Protein %
S-1	197.05a	69.27a	3.26ab	27.74bc	15.22a	27.58d
S-2	185.74a	58.20b	3.67a	29.27ab	15.28a	21.51e
S-3	157.55b	49.73b	3.50ab	30.88a	14.81a	20.95e
S-4	21.58e	6.84d	1.82c	25.35cd	9.56b	37.96c
S-5	66.43c	20.18c	2.10c	23.64de	9.55b	47.94b
S-6	23.44d	7.22d	1.96c	26.89bc	9.23b	52.85a
S-7	-*	-				
S-8	179.33ab	49.42b	2.96b	20.97e	14.74a	37.02c
S-9	-	-				
LSD p>0.01	22.248	9.022	0.595	1.357	1.923	3.511

*No data. Mean values marked with a different letter in each column are statistically different from each other

Quality parameters such as cap diameter, stem length and protein content are important in terms of marketability and nutritional value of the mushroom. The highest value in mushroom weight was determined as 3.67 g on average in S-2. Mushroom weight was determined as 3.26 g and 3.50 g on average in S-1 and S-3, respectively. The lowest mushroom weight was found in S-4 (1.82 g). Cap diameter showed significant differences according to the substrates. Cap diameter varied between 20.97-30.88 mm. The lowest and highest cap diameters were measured in S-8 and S-3, respectively. The cap diameter determined by Okuyucu (2021) and Baybas (2023) is close to the values we found with 29.69-39.10 mm and 17.94-23.70 mm, respectively, and is similar to the values found by Karasoy (2022) for cap diameter as 22.90-31.90 mm. It is known that the difference in cap diameters varies depending on environmental conditions and delay in harvest time. The longest stem was measured in S-1 (15.22), S-2 (15.28), and S-8 (14.74). These groups are statistically similar. The shortest stem was measured as 9.56 cm, 9.55 cm, and 9.23 cm in S-4, S-5, and S-6, respectively.

Enoki is a good source of protein. The protein value varied between 20.95-52.85%. The highest protein was calculated from the mushrooms cultivated on S- 6 with a rate of 52.85%. This was followed by S5 with a protein rate of 47.94% and S-4 and S-8 with protein rates of 37.96 and 37.02%, respectively. Cohen et al (2014) determined the protein rate in enoki as 23.4%.

Ash, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) values of enoki cultivated on different substrates are given in Table 5. The values in the table show the mineral concentrations of the mushroom in mg per kg dry weight.

Table 5. Mineral contents of mushrooms grown in different substrate formulations.

Substrat	Ash %	N%	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	Fe	Cu mg kg^{-1}	Zn mg kg^{-1}	Mn
S-1	9.31c	4.41d	9208.30c	36078.61b	319.25d	2014.60b	60.84c	12.51d	58.54d	6.10b
S-2	11.41a	3.44e	7194.70d	31364.87c	1034.82a	2023.60b	57.24c	7.64e	41.73e	7.43a
S-3	9.63c	3.35e	7535.97d	27581.87d	233.18e	2228.76a	60.04c	6.37e	45.33e	5.48c
S-4	11.43a	6.07c	12499.91a	39761.53a	764.60b	2122.68ab	86.66a	26.92a	133.10a	7.47a
S-5	9.21c	7.67b	10729.00b	32155.50c	492.31c	1363.98c	79.23b	19.54b	97.93b	7.36a
S-6	10.67b	8.45a	9992.92bc	36929.28b	239.19e	1313.04c	85.86a	17.91c	97.87b	7.40a
S-7	-*	-	-	-	-	-	-	-	-	-
S-8	7.01d	5.92c	7537.97d	24219.20e	277.22de	1128.90d	60.44c	12.71d	67.75c	6.63b
S-9	-	-	-	-	-	-	-	-	-	-
LCD	0.518		818.270	2664.529	53.940	136.620	6.084	1.582	7.905	0.570

*No data. Mean values marked with a different letter in each column are statistically different from each other

Ash content shows the amount of inorganic matter in the mushroom, and S-2 and S-4 stand out in this respect. The highest ash was seen in S-2 and S-4 (11.41% and 11.43%), while the lowest was seen in substrate 8 (7.01%). Cohen et al. (2014) reported the ash of enoki as 8.3%. The highest N content was found in S-6 (8.45%), while the lowest was found in S-3 (3.35%). S-6 was found to be the richest in N. Significant differences were detected among the substrates in terms of all mineral substances analysed in the harvested mushrooms. The mineral with the highest concentration in mushrooms was K (27581.87-39761.53 mg kg^{-1} dry weight) in all substrates. This shows that enoki are a rich source of K. P was found at high levels in mushrooms obtained from all substrate formulations (7194.70-12499.91 mg 100 g^{-1} dry weight). Mushrooms grown in S-4 stand out with the highest levels of many minerals (P, K, Fe, Cu, Zn, Mn). Mushrooms from S-2 and S-8, which have high yield values, had low values in terms of many minerals.

Conclusion

The results obtained emphasize that the choice of substrate in enoki cultivation directly affects the yield and production period. The nutrient content (C/N ratio), physical structure (air permeability, moisture retention capacity), incubation conditions (temperature, pH, CO_2, and sterilization) of the substrate affect the mycelial development period. S-1 and S-2 stand out with their high yield, BE ratio and stem length. However, their protein content was low. If the aim is yield; Substrates S-1 and S-2 should be preferred. S-4, S-5 and S-6 have low yield and short stem length. Protein ratios were high but mushroom weights were low. S-8 has a higher protein content compared to other high yield substrates (S-1, S-2 and S-3), but it was found to be at a medium level in terms of mushroom weight and cap diameter. S-6 had the highest protein content, but its yield and mushroom weight were found to be low. S-8 was determined as a medium level option in terms of both yield and protein. The substrate selection in mushroom cultivation should be decided according to the targeted parameters. It has been determined that local resources such as cotton gin waste are suitable for high yields, but substrate optimization is needed if protein and mineral content is to be increased. These findings provide important contributions to sustainable mushroom cultivation and the utilization of agricultural wastes. Agricultural wastes can provide economical and sustainable substrate alternatives. More controlled experiments should be conducted to compare the productivity of different substrates.

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Author Contributions:

NC; planned of overall research, statistical analyses, writing-review and editing; MKS; planned of overall research, preperad spawn, and ST; Cultivated the mushroom, prepared of samples and laboratory analysis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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