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Exploring the biochemical and productivity implications of incorporating mustard cake in *Hypsizygus ulmarius* (Bull.) cultivation

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Abstract

The study aimed to evaluate the biological efficiency and nutritional composition of *Hypsizygus ulmarius* (Bull.), an edible and medicinal mushroom species often overlooked by Indian farmers. The mushrooms were cultivated on wheat husk as the control substrate from October to February. The substrate was enriched with mustard cake (M) at concentrations of 10 % (M500), 20 % (M1000) and 30 % (M1500) by dry weight to assess the impact on yield. The growth parameters, including the size and weight of fruiting bodies from each type of substrate were recorded. The control substrate, consisting of wheat straw alone, required the shortest period for mycelium growth at 33.125 ± 0.99 days, while wheat straw supplemented with 30 % mustard cake (M1500) took the longest time at 46.6 ± 1.5 days. However, the M1000 substrate (20 % mustard cake) had the shortest time span for pin head formation, taking 46 ± 1.41days and for maturation to fully grown fruiting bodies, requiring 50.25 ± 2.12 days. The M1000 substrate also produced the highest yield of 5533.2 kg with the greatest biological efficiency of 92.2 %, but comparatively least values were obtained in M1500, with a yield of 2088.28 kg and a biological efficiency of 51.28 %. The highest total carbohydrate content was found in M500 (10 % mustard cake), at 71.91 %, along with a fiber content of 13.19 %. The highest protein (38.06 %) and fats (2.73 %) contents were observed in M1500. The results of this study suggest that mustard cake can be used as an effective supplement to enhance the nutritional content, biological efficiency and productivity of *Hypsizygus ulmarius*.

Keywords

Hypsizygus ulmarius ; biological efficiency; nutritional composition; protein; fats

Introduction

Mushrooms are low in calorie and are often referred to as 'white vegetables' or 'boneless vegetarian meat', rich in polysaccharides, proteins, vitamins and with unique medicinal properties (1). The Indian subcontinent is endowed with diverse agro-climatic zones, providing a wealth of fungal diversity (2). Despite the widespread occurrence of mushrooms in India, many Indian farmers remain unaware of the cultivation techniques and nutritional benefits of edible mushrooms (3, 4). With India surpassing China's population in 2024 and a current annual growth rate of 0.91 % (5), there is an increasing need to explore sustainable food sources such as mushrooms. There is a need to expand mushroom farming to help address food scarcity (6, 7). Mushrooms hold significant potential to boost the Indian economy due to diverse applications in nutrition, medicine and industry. Recently, the National Horticulture Board reported that Bihar was the largest mushroom-producing state in 2021–2022, with over 28000 metric tonnes produced, accounting for 10.82 % of the country's total production (8). It was followed by Maharashtra with 9.89 %, and Odisha with 9.66 % (9). Earlier, studies revealed that between 2010 and 2017, the mushroom industry grew at an annum rate of 4.3 %, with white button mushroom dominating the market at 73 %. Oyster mushrooms, paddy straw mushrooms and milk mushrooms made up 16 %, 7 % and 3 %, of the total market share respectively (10). Bihar, Maharashtra, Orissa and Haryana emerged as the leading mushroom-producing states, contributing 10.82 %, 9.89 %, 9.66 %, and 8.19 % to the country's overall production (10, 11).

The elm mushroom (*Hypsizygus ulmarius* (Bull.)), previously known as Pleurotus ulmarius, was first introduced to Indian mushroom growers by the Indian Institute of Horticulture Research (IIHR) in Bengaluru, India (12). This edible and medicinal mushroom typically grows on the trunks or logs of elm trees in forests. The mushroom has blue pinheads that turn dull white as they mature (13). Their large, palatable, gilled fruiting bodies grow in clusters, have a shelf life of 36-48 h at temperature between 25-30 °C and possess a rich, meaty flavour (14). It is well-established that the substrate used in mushrooms cultivation serves as a key source of nutrition, directly influencing their biological efficiency, nutritional and medicinal values and the ability to degrade lignocellulose substrates (15). Therefore, to achieve maximum yield and enhanced nutritional value, it is essential to select or prepare a suitable substrate for mushroom cultivation (16). Researchers have been investigating variations in mushroom yield, both quantitatively and qualitatively, under different environmental conditions and using various substrates (17-22). Numerous organic wastes, such as bean straw, corn silage, wheat straw, popular sawdust and pine sawdust, have been used to study (23) the rate of lignocellulose degradation by *Hypsizygus ulmarius* and its effects on growth. Their study revealed that bean straw could enhance yield and shorten the crop cycle of this mushroom species. Other studies investigating the best combination of substrates (14) have used wheat straw, paddy straw, cardboard and banana leaves in various proportions to obtain maximum yield, biological efficiency and nutritional value for *Hypsizygus ulmarius*. Despite these efforts, there is significant potential to explore mushroom cultivation behaviour using certain agricultural waste (24). The present study aims to identify the best alternative substrate that supports the growth of elm oyster mushrooms, yielding high crop output, larger fruit sizes and a manageable crop cycle for Indian farmers. The research involved preparing different combinations of wheat straw as the primary substrate, supplemented with mustard cake (M), to evaluate their effects on biological efficiency and cultivation patterns.

Materials and Methods

The mycelium (spawn) of *H. ulmarius* mushrooms was obtained from the IIHR, Bangalore, India. The spawn, which is the vegetative growth of the fungus, was used to inoculate the substrate and initiate the cultivation process. 5 kg of milled wheat straw were soaked for 6-7 h, air-dried and thoroughly mixed with coarse mustard cake powder at concentrations of 10 % (500 g), 20 % (1000 g) and 30 % (1500 g) by dry weight, creating 3 substrate-supplement combinations. The bags were labelled to indicate their respective concentrations as M500, M1000, M1500 and control. Propylene bags (60 cm × 30 cm) were filled with the prepared substrates and autoclaved at 15 PSI and 121 °C for 20 min. After autoclaving, the substrates were inoculated with the spawn at a rate of 5 % (w/w) of the total substrate-supplement weight. Sterile needles were used to perforate the bags, creating 12–15 holes (pinning) to allow aeration. The bags were then incubated for the mycelium run, placed on iron shelves in complete darkness at a temperature of 25-30 ºC. The number of days required for the mycelium to run in each substrate type was recorded. Once the bags exhibited full white mycelial growth, they were transferred to cropping rooms with controlled conditions-temperature (25-30 ºC), humidity (70-80 %) and light-until fully developed fruiting bodies (mushrooms) were harvested.

The duration of primordial body formation was defined as the period between spawning and pinhead formation. The time taken for the fruiting body to mature was measured as the number of days from spawning to the first harvest. The numbers of fully grown fruiting bodies per bag was recorded and the weight and size of the pileus and stipe were measured separately. Biological efficiency was calculated using the following formula (25).

$$\text{Biological efficiency (BE \%)} = \frac{\text{Fresh weight of harvested mushroom}}{\text{Dry weight of the substrate}} \times 100$$

The harvested mushrooms were oven dried at 45-55 °C (26). The dried fruiting bodies were then extracted using petroleum ether and ethanol. The organic solvents were evaporated from the extracts and the resulting extracts were stored in labelled eppendorf tubes at 4 °C for further analysis.

Parameters such as moisture, volatile content and ash percentage were analyzed using standard methods (27-29). Nutritional analysis of the collected fruiting bodies, including carbohydrates, proteins, fat, fibre and energy (kcal/100 g), was also conducted (28, 30). The total protein content was estimated using the Micro Kjeldahl method (30). The energy obtained from 100 g of dried mushroom was calculated using the formula (31):

$$\text{Energy (kcal/100 g)} = \text{Total carbohydrates} \times 4 + \text{fat} \times 9 + \text{protein} \times 4$$

Data were collected in triplicates and Fisher's analysis of variance was applied to assess the overall significance of the data. Significant differences among treatment means were compared using the LSD- Least Significant Difference Test at P≤0.05 (32).

Results and Discussion

Influence of substrate- supplement combination on mushroom crop cycle

The study found that the addition of mustard cake increased the duration of mycelium running and primordial body formation, but fruit development occurred more quickly compared to the control. There were significant differences ($p < 0.05$) in the mycelium run, pinhead formation and first harvest periods among the different levels of mustard cake supplementation (Table 1). The control substrate, which contained only wheat straw and no supplements, had a mycelium run period of 33.12 ± 0.99 days, pinhead formation at 38.75 ± 1.66 days and a significantly longer first harvest period of 58.8 ± 1.60 days. With the addition of 10 %, 20 % and 30 % mustard cake, the mycelium run periods were extended to 35.57 ± 1.39, 40.25 ± 1.66 and 46.6 ± 1.5 days respectively. Previous observations of *Hypsizygus ulmarius using* different substrates have shown that the duration of mycelium run varies with substrate type, ranging from 19.1-31.1 days, which is consistent with the findings for the Control and M500 (10 %) in this study (23). Similar to the current results, earlier studies reported significant variation in the duration of mycelium run for *Hypsizygus ulmarius*; sugarcane bagasse resulted in the shortest time at 16.50 days, while groundnut hulls required the longest at 23.75 days (33). However, other research has indicated that *Hypsizygus ulmarius* on wheat straw took only 14.50 days for mycelium to run (14).

The formation of primordial bodies or pinheads took longer in the supplemented substrates, with durations of 48.71 ± 3.35, 46 ± 1.41 and 51.33 ± 1.21 days for M500, M1000 and M1500 respectively, compared to 38.75 ± 1.66 days in the control. The study indicated that primordial initiation or pinhead formation ranged from 32.6 to 50.6 days (23), which aligns with the present study's maximum duration of 51.33 ± 1.21 days. Although some earlier reports noted a shorter pinhead formation period of 20.33 to 28 days (34), this variation could be attributed to differences in the lignin and cellulose content of the substrates. Mature fruiting bodies were harvested after 58.8 ± 1.60 days from non-supplemented substrates, which was the longest period observed among all samples. In contrast, the M1000 (20 % mustard cake) substrate yielded the shortest fruit development period of 50.25 ± 2.12 days. All observations for the first harvest fell within the previously reported range of 39.8 to 62.0 days for various substrates (23) (Fig. 1).

Influence of substrate- supplement combination on total yield, number of effective fruiting bodies (NEFB) and biological efficiency (BE %)

The study found that adding mustard cake as a supplement to the substrate had a significant impact on the yield and biological efficiency of the mushrooms. Total yield ranged from 2088.28–5533.20 g, with BE percentages ranging from 51.82 % to 92.2 % for 30 % and 20 % mustard cake supplementation respectively (Table 1). The 20 % supplement (M1000) also showed a notable improvement in yield and biological efficiency, achieving an efficiency of 84.33 %. These results are consistent with previously reported BE percentage ranging from 36.0 to 93.1 % (23). Comparable BE percentages of 90.10 % and 77.60 % have been recorded using paddy straw (33, 35). Another study reported a maximum biological efficiency of 84.11 % with neem cake as a supplement and a minimum BE of 32.00 % with soybean flour (34). Few Indian researchers have studied various agro-wastes as substrates for *Hypsizygus ulmarius* Co $(OM)_2$ strain and reported maximum biological efficiency of 99.8 % with paddy straw and water hya-

Table 1. Effect of varying concentrations of mustard cake supplement on growth, harvesting time, total yield, number of effective fruiting bodies (NEFB) per bag, biological efficiency, proximate analysis and nutritive composition of *Hypsizygus ulmarius*.

Sl. No.		Supplement concentration					
		M500	M1000	M1500	Control	Mean	SD
1	Mycelium run (days)	35.57±1.39^a	40.25±1.66^b	46.60±1.5^c	33.12±0.991^a	38.885	5.933209
2	Pin head formation (days)	48.71±3.35^b	46.0±1.41^a	51.33±1.21^b	38.75±1.66^b	46.1975	5.420931
3	First harvest (days)	52.57±4.27^c	50.25±2.12^b	58.33±5.60^a	58.80±1.60^c	54.9875	4.242471
4	Total yield (g)	4638.53±2.37^a	5533.2±6.07^c	2088.28±2.57^b	3383.6±3.07^b	3910.903	1501.254
5	NEFB per bag	48.57±6.5^c	37.60±7.4^a	15.80±7.7^c	38.12±7.41^a	35.0225	13.77531
6	Biological efficiency (%)	84.33±1.7^b	92.2±9.5^b	51.82±8.5^b	67.67±4.32^b	74.005	17.98119
7	Moisture content (%)	93.05±4.1^a	91.58±7.5^a	87.75±3.5^a	83.05±8.02^a	88.8575	4.469999
8	Ash content (%)	0.98±9.6^c	0.48±0.5^b	0.97±0.53^c	0.19±0.56^b	0.655	0.388029
9	Volatile content (%)	2.03±0.2^b	2.9±0.5^c	2.06±0.52^b	2.7±1.71^c	2.4225	0.44365
10	Total Carbohydrates (%)	71.91±1.01^a	61.33±1.5^a	58.24±4.5^c	69.7±3.43^b	65.295	6.548702
11	Protein (%)	25.16±2.03^c	36.57±3.5^b	38.06±2.5^a	28.27±4.54^a	32.015	6.279758
12	Crude fat (%)	2.07±1.05^a	2.62±4.5^c	2.73±0.6^b	2.01±0.21^c	2.3575	0.370169
13	Fiber content (%)	13.19±0.56^a	8.75±2.5^a	10.98±5.5^c	12.41±3.43^b	11.3325	1.949776
14	Energy (kcal/100 g)	406.91±0.71^c	406.18±5.78^b	409.77±7.71^b	409.98±9.01^c	408.21	1.947426

$p < 0.05$ level of significance using Duncan's multiple range test (DMRT).

Fig. 1. Showing growth of *Hypsizygus ulmarius* mushroom at different concentrations of mustard cake. (**A**) control, (**B**) M500, (**C**) M1000 and (**D**) M1500 concentration.

cinth and a minimum of 49.4 % with coir pith (36). Given that Uttarakhand does not produce much water hyacinth waste, this study focuses on local agro-waste like mustard cake and wheat straw combinations. However, a previous report indicated higher efficiency ranges of 155.60 % and 136.88 % (14) and another study found extremely high BEs of 231.7 % and 437.5 % using wheat straw and biogas digester liquid-sprayed wheat straw respectively (37). A related study on the king oyster mushroom (*Pleurotus eryngii*) reported a biological efficiency of 48.24 % with wheat straw, compared to 51.82 % in the control sample of this study (38). The study also found that a 30 % mustard cake supplement (M1500) significantly reduced both yield and biological efficiency (Table 1). These findings suggest that while a 20 % mustard cake supplement can significantly improve yield and biological efficiency, the 30 % supplement may not be optimal due to their high lignocellulose content.

The number of effective fruiting bodies (NEFB) per bag was highest with the 10 % mustard cake supplement, averaging 48.57 ± 6.5 and lowest with 30 % supplemented, averaging 15.8 ± 7.7, indicating that M500 supports better development of fruiting bodies. Previous studies have reported a wide range of fruiting bodies per bag from 6.11 to 89.56 (34).

Influence of substrate- supplement combination on size of fruiting bodies

The study observed that the largest pileus-size fruiting bodies, measuring 8.40 cm, were obtained from the M1500 beds, while the smallest, at 7.5 cm, came from the M1000 beds. The M1000 (20 % mustard cake) supplementation resulted in the tallest stipe at 5.9 cm, whereas the M1500 (30 % mustard cake) had the shortest stipe at 4.9 cm. The maximum stem diameter was 0.91 cm for fruiting bodies from M1000, while the minimum was 0.71 cm from M500. A recent study reported a maximum pileus size of 6.72 cm with 2 % karanja cake supplementation and a minimum size of 4.09 cm with 2 % soybean flour supplementation. The maximum stipe length was 4.43 cm with 2 % wheat supplementation, while the minimum was 2.62 cm with 2 % karanja cake supplementation. Additionally, the maximum stipe diameter was 3.84 cm with the addition of 2 % g flour and the minimum was 3.19 cm on wheat straw as the control (34) (Table 2). Researchers have reported that differences in pileus width may result from the substrate material, which affects stalk height, stalk diameter and cap size in mushrooms (39). In their study, the maximum stipe length of 11.17 cm was observed with T2 (wheat straw + bajra dry stem), while the minimum length was 7.50 cm with T1 (wheat straw + sarpat). The maximum pileus width in T2 was 8.33 cm and the minimum was 5.67 cm in T1 (39). Another report indicated that the maximum cap or pileus diameter on corn husk substrate was 7.9 ± 3.5 cm, whereas the minimum on paddy straw was 6.63 ± 0.66 cm. The maximum stipe diameter on a combination of corn husk and paddy straw was 1.6 ± 0.55 cm, with a minimum stipe diameter of 1.06 ± 0.11 cm and a maximum stipe length of 4.36 ± 1.01 cm on corn husk substrate (40).

Table 2. Effect of supplemented substrate over the size of fruiting bodies of *H. ulmarius.*

Sl. No.	Cultivation bag code	Diameter of pileus (cm)	Length of stipe (cm)	Diameter of stipe (cm)
1	M500	8.35^{b}	5.5^{a}	0.71^{b}
2	M1000	7.5^{c}	5.9^{bc}	0.91^{bc}
3	M1500	8.4^{bc}	4.9^{a}	0.83^{a}
LSD		0.01	0.002	0.032
CV		6.25	9.26	12.32

*Values in the same column followed by a different lowercase letter are significantly different at P< 0.05; LSD- Least Significant Difference Test at P≤0.05; CV- Coefficient of Variance

Influence on proximate analysis and nutritive composition of fruiting bodies harvested from varying supplemented bags

The ash content was highest in fruiting bodies from M500 bags, at 0.98 %, followed by M1500 at 0.97 %, M1000 at 0.48 % and the control at 0.19 %. Several studies have reported significantly higher ash contents, ranging from 4.3 % to 13.06 % (30, 38, 40-44). The volatile content was highest in mushrooms grown on M1000 at 2.9 % and lowest in M500 at 2.03 %. The volatile contents for M1500 and the control substrates were 2.06 % and 2.70 % respectively. The proximate analysis of *Hypsizygus ulmarius* grown on supplemented substrates was compared to the control substrate (Table 1). The moisture content was lowest in the control at 83.05 % and highest in M500 at 93.05 %, with M1500 showing a moisture content of 87.75 %. Studies on various edible mushrooms, including both fruiting bodies and submerged cultures, report moisture content ranging between 85 % and 95 % (28). Another study using the same strain of *Hypsizygus ulmarius* (IIHR Hul) found a comparable moisture content of 90.06 % (41). For related species like *Pleurotus eryngii*, moisture content ranged from 85.10 % on sugarcane bagasse to 88.66 % on wheat straw (38). Literature shows moisture content ranges from 83.05 % to 91.8 % (28, 42), while another study reported a range of 61.66 % to 69.86 % (40). Variations in moisture content can be attributed to differences in substrate, fungal strain, substrate bed moisture and environmental conditions during cultivation.

Proximate nutritive composition

The protein percentage in the mushrooms ranged from 25.16 % to 38.06 %, reflecting the increased concentration of supplementation. A recent study on the same species reported a lower protein percentage of 12.85 % to 17.00 % with various supplements at different concentrations (44) (Table 1). In another study of various edible mushrooms, the protein concentration ranged from 8.60 % in *Trametes versicolor* to 38.7 % in *Ophiocordyceps sinensis* (28). Research on the same species with seaweed supplementation found that crude protein concentration increased in all fruiting bodies compared to the control substrate, with values ranging from a minimum of 17.37 % to a maximum of 21.22 % (30).

The total carbohydrate percentage in the mushrooms varied from 58.24 % in M1500 to 71.91 % in M500. A recent study reported carbohydrate content ranging from 66.54 % (with 30 % maximum supplementation) to 75.15 % (with 10 % minimum supplementation), which is in agreement with the current findings (44). Another study found that the maximum soluble carbohydrate content in the control sample without supplementation was 11.91 %, while seaweed supplementation at 5 %, 10 % and 20 % resulted in carbohydrate contents of 10.53 %, 10.13 % and 5.75 % respectively (34). Additionally, the total carbohydrate percentage in most edible and medicinal Basidiomycetes mushrooms typically ranges from 42.9 % to 83.5 % (28). In contrast, another study reported a lower carbohydrate content of 32.00 % to 47.81 % for the same species (40).

The crude fat percentage was highest in *Hypsizygus ulmarius* fruiting bodies harvested from M1500 at 2.73 %, followed by M1000 at 2.62 %, M500 at 2.07 % and the control at 2.01 %. The study observed a broader range of lipid content in edible mushrooms, from 0.8 % to 7.9 %. In *H. ulmarius*, a similar fat range of 1.7 % to 3.50 % has been reported (28, 30, 36). For *H. ulmarius* grown on seaweed-alternated rice straw substrates, the fat content ranged from 1.48 % to 1.98 %. Some studies have reported even higher fat contents, ranging from 4.80 % to 10.03 % and up to 26.20 % (40, 41). The fiber content was highest in fruiting bodies from M500 at 13.19 %, followed by the control at 12.41 %, M1500 at 10.98 % and M1000 at 8.75 %. These values are lower than previously reported ranges of 15.75 % to 19.42 % and 15.45 % to 19.45 % (30, 41).

Conclusion

According to our findings, mustard cake can be used as a significant supplement to enhance yield, biological efficiency and nutritional content. The integration of mustard cake was observed to increase the time required for mycelium run and pinhead formation, but it promotes the faster development of fruiting bodies, which helps in obtaining flushes earlier as it shortens the crop cycle. The biological efficiency, protein content and crude fat percentage of *H. ulmarius* were positively correlated with the concentration of mustard cake supplementation. However, while the total carbohydrate percentage decreased with higher mustard cake supplementation, both protein and fat percentages increased. Therefore, a 20 % mustard cake supplementation (M1000) is recommended for optimal yield and nutritional value in *H. ulmarius* cultivation.

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Authors' contributions

RC conducts experiments and initial draft of the manuscript. The experiment's framework was created by SR, RA and RK after retrieving research and review publications. The statistical analysis was carried out and the results were compiled by JCP, MT and PKC. Technical help is given by SK, who also edits the manuscript and IR Finalize manuscript for publishing form and oversees the entire work as supervisor.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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