

Effect of Different Saw Dust Substrates on the Nutritional Composition of Oyster Mushroom (*Pleurotus florida*) and its Applications in Human Health

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ABSTRACT: This study evaluated the effects of various sawdust substrates, namely *Ficus carica* (Fig tree, T₂), *Albizia saman* (Rain Tree, T₃), *Swietenia mahagoni* (Mahogany tree, T₄), *Leucaena leucocephala* (Ipil ipil tree, T₅), *Eucalyptus oblique* (Indian gum tree, T₆) and mixture of mentioned five trees sawdust (T₁) supplemented with 30% wheat bran and 1% lime on the mineral content and nutritional composition analyses of *Pleurotus florida* mushroom. The highest amount of carbohydrate (43.68mg/100g), dry matter (9.87%), nitrogen (4.43%), potassium (1.39%), magnesium (19.96mg/100g) and iron (44.69mg/100g) were obtained from T₄ sawdust substrate. The highest amount of phosphorous (0.89%) was obtained from T₁ substrate. The highest amount of lipid (4.21%), protein (27.68%), ash (13.67%), moisture (90.27%) and molybdenum (9.76mg/100g) were obtained from T₂ sawdust substrate. The lowest amount of lipid (3.42%) was found for T₆ substrate. The highest amount of crude fiber (19.78%), calcium (33.35mg/100g) and selenium (9.10 mg/100g) were obtained from T₅ substrate. The highest amount of cobalt (21.07mg/100g) and zinc (30.26mg/100g) were obtained from T₃ sawdust substrate. Thus, they could be an excellent source of many different nutraceuticals and might be used directly in human diet to promote health for the synergistic effects of all the bioactive compounds present. The study proved beneficial for effective management of agricultural wastes as well as production of nutraceutical effective fruiting bodies for balanced diet.

Key words: *Pleurotus florida*, nutrition; proximate analysis, mineral content, saw dust, Wheat Bran

INTRODUCTION

Oyster mushrooms are edible fungi and belong to the genus *Pleurotus*, class-Basidiomycetes, order-Agaricales and family-Pleurotaceae.^{1,2} For the excellent flavor and taste *Pleurotus* species are delicacies in different parts of the world.³⁻⁷ Cultivation of the mushroom can create working area

and eradicate unemployment problem by creating employment opportunity in urban and rural areas.⁸ These mushrooms can also ensure the availability of it at low price and for recycling of agricultural wastage.⁹⁻¹¹

Since time immemorial mushrooms are being used as food and medicine to promote good health and vitality and increasing body's adaptive abilities.¹² More than 100 medicinal functions are produced by mushrooms and the key medicinal uses are

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antioxidant, anticancer, antidiabetic, immunomodulating, cardiovascular protector, antiviral, antibacterial, antiparasitic, detoxification and hepatoprotective effects; they also protect against tumor development and inflammatory processes.¹³⁻¹⁷ Numerous molecules synthesized by mushrooms are known to be bioactive, and these bioactive compounds found in fruit bodies, cultured mycelium, and cultured broth are polysaccharides, proteins, fats, minerals, glycosides, alkaloids, terpenoids, tocopherols, phenolics, flavonoids, carotenoids, lectins, enzymes, ascorbic, and organic acids, in general. Polysaccharides are the most important for modern medicine and α -glucan is the best known and the most versatile metabolites with a wide spectrum of biological activities.^{13,15,18,19,22} They have also a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fiber, poor fat but with excellent important fatty acids content. Moreover, edible mushrooms provide a nutritionally significant content of vitamins (B₁, B₂, B₁₂, C, D, and E).^{20,21} It is important to note that all *Pleurotus* species are consumable.^{7,14,23-27}

Oyster mushrooms are commercial mushrooms that are least expensive and easiest to grow because they are well known for conversion of crop residues to food protein.²⁸ A number of studies on cultivation of them on solid substrates such as sawdust and different agricultural wastes such as rice bran, wheat bran, sugarcane bagasse, rice husks, coconut fiber, peanut hulls, and banana leaves etc.²⁹⁻³² to improve the production, quality, flavor, and shelf life of cultivated mushrooms have been reported.³³⁻³⁵

Sawdust, a by-product of the saw-mill industries, is largely available and has been considered a possible alternative for mushroom cultivation. Additional nitrogen, phosphate and potassium are required for the cultivation of mushrooms with sawdust as the ligno-cellulosic materials in sawdust are generally low in protein content and thus insufficient for the cultivation of mushrooms. Prior to spawning for enhancement of the yield of mushrooms supplementation of the substrate with various materials is thus recommended.³⁶ The use of wheat

and other cereal bran, as supplements, has gained importance in the cultivation of mushrooms. Wheat bran is rich in protein (~14%), carbohydrates (~27%), minerals (~5%), fat (~6%) and B vitamins.³⁷

For this potential and nutritive edible item, works on the nutritive analysis are not available in Bangladesh and there is no mushroom based balanced diet charts for the common people as well as for the patient. For this reason the proximate analysis for the oyster mushroom (*Pleurotus florida*) is necessary. This is also important to find out the nutritional status of mushroom growing in different substrates, which will help to select mushrooms as a food in balanced diet. Therefore, the objective of this work is to find out the suitable saw dust with substrate for proximate analysis and determination of mineral contents of oyster mushroom (*Pleurotus florida*).

MATERIALS AND METHODS

Experimental location and materials. The experiment was carried out jointly at the Mushroom Culture House (MCH), Biochemistry laboratory of the Department of Biochemistry, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh and Material Chemistry laboratory of Department of Chemistry, BUET, Dhaka-1000, Bangladesh. Fruiting body of *Pleurotus florida* was collected from National Mushroom Development and Extension Center (NAMDEC), Saver, Dhaka, Bangladesh. *Pleurotus florida* was grown on different saw dust supplemented with 30% wheat bran and 1% lime on the spawn packet. All the chemicals used were collected from Merck (Germany), Wako Pure Chemicals Industries Ltd. and JHD (China). The sample was weighted by electric balance (KEY: JY-2003; China) and heated in a muffle furnace (Nebertherm: Mod-L9/11/c6; Germany). The amount of minerals were determined by atomic absorption spectrophotometer (analytikjenanov AA 400P; Germany), flame photometry (PFP7; Germany), and spectrophotometer (HALO BD-20S; Germany).

Cultivation and harvesting of *Pleurotus florida* mushroom. Five different saw dust substrates

namely, fig tree (*Ficus carica*, T₂), rain tree (*Albiziasaman*, T₃), mahogany tree (*Swietenia mahagoni*, T₄), ipil ipil tree (*Leucaenaleucocephala*, T₅), Indian gum tree (*Eucalyptus obliqua*, T₆) and a control (T₁) supplemented with 30% wheat bran and 1% lime were used throughout the present study. Sterilization, cultivation, and harvesting of the oyster mushroom (*Pleurotus florida*) were performed on spawn packets following literature.³⁸⁻⁴⁰ Then the harvested mushroom samples were subjected to proximate and mineral content analyses.

Proximate analysis. Mushrooms grown from the spawn were collected from each packet separately and all the wastes and dusts were removed from the fruiting body. Thereafter, they were ready to analyze.

Estimation of moisture. About 10-20 g of the material of each sample were weighed separately and then the samples were dried in an oven at 105 °C with pre-weighed petridishes till the weight of the petridishes with their contents were constant. The moisture content was expressed by percent and evaluated by the formula described by Raghuramulu *et al.*, 2003 and Sarker *et al.*, 2007.^{41,42}

Estimation dry matter. A clean container (dish or beaker) was place in an oven at 105 °C overnight. The container was allowed to cool in a desiccator and was weighted. The samples were kept into the container and the samples were weighted. The container was placed in the oven at 105 °C for 24 h again. The container was allowed to cool in a desiccator and was weighted. Again, the container was placed in the oven at 105 °C for 2 h. It was cooled in a desiccator and weighted again. Repeat drying, cooling and weighing was continued until the weight becomes constant. Then the percentage of dry matter content of the sample was evaluated by the formula found elsewhere.^{41,42}

$$\text{Moisture (\%)} = \frac{\text{Initial Wt. - Final Wt.}}{\text{Original weight of sample}} \times 100$$

$$\text{Dry Matter (\%)} = \frac{\text{Wt. an of oven dried sample}}{\text{Original weight of sample}} \times 100$$

Estimation of crude fiber. Ten grams of moisture and fat-free sample was taken in a beaker

and was added 200 ml of boiling 0.255 N H₂SO₄. The mixture was boiled for 30 min to keep the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH added. After boiling for 30 min (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100 °C and weighed. The crucible was heated in a muffle furnace at 600 °C for 5-6 h, cooled and weighed again. The difference of those two mentioned weights represents the weight of crude fiber and percentage was calculated by the formula found in the literature.^{41,43}

$$\text{Crude Fiber (\%)} = \frac{\text{Dry weight after digestion(W}_d\text{)} - \text{Weight of ash(W}_a\text{)}}{\text{weight of moisture and fat-free fat-free sample}} \times 100$$

Estimation of lipid. Fat was estimated by crude ether extraction of the dry materials. The dried samples (about 5g) were weighted into a conical flask and plugged with fat free cotton. The flask was then placed in an electric shaker and extracted with anhydrous ether for about 16 h. The ether extract was filtered into another weighed conical flask. The flask containing the original ether extract was washed 4 to 5 times with small quantities of ether and the washings were also transferred to the filter paper. The ether in the conical flask was then removed by evaporation, and the flask with the residual was dried in an oven at 80 °C to 100 °C, cooled in a desiccator and weighted as well as percentage of lipid was determined by the formula found elsewhere.⁴¹

$$\text{Fat Contents (g) per 100g of Dried Sample} = \frac{\text{Weight of Ether Extract} \times \text{Percentage of Dried Sample}}{\text{Weight of Dried Sample Taken}}$$

Estimation of total carbohydrate: Carbohydrate content was determined and expressed by percentage by the formula found in the literature.^{41,44}

$$\text{Carbohydrate (g/100g sample)} = 100 - [(\text{Moisture} + \text{Fat} + \text{Protein} + \text{Ash} + \text{Crude Fiber}) \text{ g/100g}]$$

Estimation of total ash: Ash content of the samples was determined by heating the pre dried samples in a muffle furnace for about 5-6 h at 600 °C until stable weight is gained and white or grayish white color is obtained. Percentage of ash also calculated by the formula found elsewhere.⁴¹

$$\text{Ash content (g/100g sample)} = \text{Wt of ash} \times 100 / \text{Wt of sample taken}$$

Estimation of crude protein: Sample was dried and grinded using a mortar and pestle to analyze crude protein content. James (1995) and Chang & Buswell (2003) described the Kjeldahl method in

which the nitrogen content was first determined and then multiplied with 6.25 to obtain the protein content of the sample.^{45,46} Then the percentages of protein were calculated by using the formula.

$$\text{Crude protein (\%)} = \% \text{ N} \times 6.25$$

Mineral content estimation: Total nitrogen was determined by a micro kjaldhal apparatus in the traditional method and calculated using the following formula.

$$\% \text{ N in the supplied fiber sample} = \frac{(a \times M_{\text{HCl}} - b \times M_{\text{NaOH}}) \times 1.401}{c}$$

Where,

a = ml HCl measured into the conical flask in the distill H₂O (usually 20.00 ml)

b = ml NaOH used for titration of the content in the conical flask

c = g powder of sample used for the analysis

M_{HCl} = molarity of the HCl measured into the conical flask

M_{NaOH} = molarity of the NaOH used for titration

The sample were digested with nitric acid to release of Ca, Mg, K, Fe, and P, Ca, Mg, Fe, were determined by atomic absorption spectrophotometer, K was determined by flame photometry, and P was determined by spectrophotometer using the formula found in the literature.⁴⁷

Fe, Mo, Zn, Co and Se contents were also measured by atomic absorption spectrometer (AAS) directly in the undiluted filtrate by using the formula found in the literature.^{22,47}

$$\text{For Ca, Mg, K and P mg per kg sample} = \frac{a \times 25000}{b \times c}$$

Where,

a = mg/L Ca, Mg, K or P measured on atomic absorption spectrometer, flame photometer

b = ml diluted filtrate transferred into the 50 ml volumetric flask for determination of Ca, Mg, K and P

c = g sample weighed into the digestion tube

$$\text{For Fe, Mo, Zn, Co and Se mg per kg sample} = \frac{d \times 100}{c}$$

Where, d = mg/L Fe, measured on atomic absorption spectrophotometer c = g sample weighed into the digestion tub

Statistical analysis of data. Data on various parameters were analyzed by following standard statistical method utilizing Microsoft Office Excel 2013. These dates were analyzed by considering 5 treatments with 3 replications and 1 spawn packets in each replication. The data for the characters considered in the present experiments were statistically analyzed by the complete randomized design (CRD) and randomized complete block design (RCBD) method. The analysis of variance was conducted and means were compared following least significant difference (LSD) test at 1% and 5% level of probability for interpretation of results were the formula found in the literature.⁴⁸

RESULTS AND DISCUSSION

Proximate composition of *Pleurotus florida*.

All the treatments were statistically similar but varied numerically with each other as shown in table 1. The amount of moisture content ranged from 90.27 to 90.13% was found. The highest moisture content (90.27%) was observed under T₂ followed by T₆. However, the lowest moisture (90.13%) was in observed under T₄ treatment (Table 1). The results of the present study are supported by other such studies as Moni *et al.* (2004) found 88.15 to 91.64% moisture content. Alam *et al.* (2007) reported 87 to 87.5% moisture content for oyster mushrooms grown on different substrates^{49,22}

The amount of dry matter of fruiting body was found in 9.87 to 9.73% ranges. The highest amount

of dry matter (9.87%) was observed under T₄ followed by T₁ and T₅. The lowest amount of dry matter (9.73%) was observed under T₂ as shown in table 1. The result of the present study matches the findings of Ahmed *et al.* (2013).⁴⁴ They found dry matter of fruiting bodies ranged from 9.40-9.98 % when sawdust supplemented with cow dung.

The protein content varied from 27.68 to 25.06 %. The highest amount of protein (27.68%) was found from T₂ and the lowest amount of protein content (25.06%) was found from T₃ (Table 1). The result of Chang *et al.* (1981) corroborates the results of this present study.⁵⁰ They reported that the fruit bodies of oyster mushrooms contained 26.6-34.1% protein. Similar result was also reported by Zhang-Rui Hong *et al.* (1998).⁵¹

The lowest amount of lipid (3.42%) was counted under T₆ followed by T₁ and the highest lipid (4.21%) amount was calculated under T₃ as shown in table 1. The result of the present study was significantly varied with the findings of Chang *et al.* (1981) who found 1.1-8.0 %.⁵⁰ However this present study results well fits with the results reported by Alam *et al.* (2007), who reported 4.30-4.41% lipids in oyster mushroom grown on different substrates.²²

The highest amount of crude fiber (19.78%) was found from T₅ followed by T₃ and the lowest crude fiber (16.92%) amount was counted under T₄ as shown in table 1. The findings are corroborated by the study of Alam *et al.*, (2007), who reported 8.7 g/100g to 23.29 g/100g of fiber in *Pleurotus spp.*²²

Table 1. Effect of sawdust substrates on chemical composition of *Pleurotus florida*.

Treatments	Moisture (%)	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	CHO (%)	Crude fiber (%)
T ₁	90.17b	9.83a	26.61ab	3.43d	12.33ab	39.86bc	17.79ab
T ₂	90.27a	9.73c	27.68a	4.07a	13.67a	37.20c	17.42b
T ₃	90.20b	9.80b	25.06c	4.21a	9.33c	41.68ab	19.72a
T ₄	90.13c	9.87a	25.89bc	3.85bc	9.67c	43.68a	16.92c
T ₅	90.17b	9.83a	25.81bc	3.64cd	10.00c	40.68ab	19.78a
T ₆	90.23a	9.77b	26.09b	3.42d	10.67bc	40.13bc	19.70a
CV (%)	0.1%	5.98%	12.75%	4.48%	8.77%	2.48%	1.53%
LSD _(0.05)	0.20	0.84	4.31	0.30	1.75	1.30	0.89

Means followed by same letter significantly different at 1% or 5% level of significance.

Different saw dust substrates and wheat bran had an effect on the approximate composition of *pleurotus florida* which was shown in figure 1.

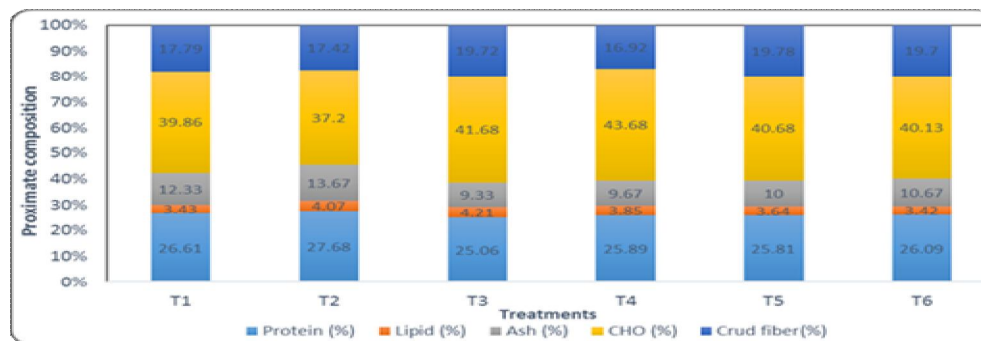


Figure 1. Effect of saw dust substrates with wheat bran on the proximate composition analysis of dry matter of *Pleurotus florida*.

The lowest amount of carbohydrate (37.20) was counted under **T₂** and the highest carbohydrate content (43.68) was found from **T₄** (Table 1). The findings of the present study does not match with the study of Chang *et al.* (1981) reported that the fruiting bodies mushrooms contained 40.30-50.7% of carbohydrates⁵⁰. But it was supported by Alam *et al.* (2007) who found 39.82 to 42.83% of carbohydrates in *Pleurotus spp.*²²

The highest ash content (13.67) was observed under **T₂** and the lowest amount of ash (9.33) was observed under **T₃** (Table 1). The findings are supported by the study of Khlood *et al.*, 2005, who reported the ash contents to be moderate in the fruiting bodies.⁵² Alam *et al.*, 2007 reported 8.28 to 9.02 % of ash in *Pleurotus spp.*²² In the present study the ash content is as high as 13.67 may be due to the newly introduced varieties.⁴⁴

Mineral contents of *Pleurotus florida*. Table 2 shows the mineral contents found from the harvested mushrooms grown under different substrate treatment along with the supplements. The highest percentage of nitrogen (4.38) was found from **T₄** followed by **T₂** (4.38) and the lowest percentage of nitrogen (4.09) was counted under **T₃** as shown in table 2. Moni *et al.*, 2004 who analyzed for various nutritional parameters and found 4.22 to 5.59% of nitrogen on dry matter basis in fruiting bodies of oyster mushroom that supports our findings.⁴⁹

The highest percentage of phosphorus (0.89) was observed under **T₁** and the lowest percentage of phosphorus (0.83) was counted under **T₃** as shown in

table 2. The finding does not match with the study of Chang *et al.*, 1981.⁵⁰ This may be due to the system of measurement. But Sarker *et al.*, 2007 found 0.97% phosphorus, in oyster mushroom grown on sawdust based substrates and which supports our findings.⁴²

Potassium percentage was found highest from **T₄** (1.39) and the lowest percentage of potassium were counted under **T₁** and **T₆** (1.17) as shown in table 2. The finding of the present study conforms to that reported by Chang *et al.*, 1981⁵⁰. They reported that the fruiting bodies of *Pleurotus* contained 1.432 to 1.88 mg/g of K on dry weigh of fruiting bodies. Sarker *et al.*, 2007 also found 1.3% potassium, in oyster mushroom grown on sawdust based substrates.⁴²

Calcium percentage was observed highest under **T₅** (33.35) and the lowest percentage of calcium was counted under **T₂** (27.66) as shown in table 2. The findings fit with the study of Alam *et al.* (2007) who found 22.15 to 33.7 mg/100g of calcium in different oyster mushroom varieties.²²

The highest percentage of magnesium (19.96) was evaluated under **T₄** and the lowest percentage of magnesium were counted under **T₂** (13.68) (Table 2). The result reported by Alam *et al.* (2007) supports our findings, who found 13.4 to 20.22 mg/100g of magnesium in different oyster mushroom varieties.²²

The highest percentage of iron (44.69) was estimated under **T₄** and the lowest percentage of iron (40.71) was counted under **T₃** as shown in table 2. Alam *et al.* (2007) reported similar result supporting our result.²² They found 33.45 to 43.2 mg/100 g of

iron in different oyster mushroom varieties. Sarker *et al.* (2007) found 92.09 ppm to 118.40 ppm iron, in oyster mushroom grown on sawdust based substrates.⁴²

The highest amount (mg) of zinc (30.26 mg/100g) was counted under **T₃** and the lowest amount (18.70 mg/100g) was of zinc counted under **T₅** as shown in table 2. The result of the present study matches with the study of Alam *et al.* (2007) found 16 to 20.9 mg/100g of zinc in different oyster mushroom varieties.²² Sarker *et al.* (2007) found

30.92 ppm zinc, in oyster mushroom grown on sawdust based substrates.⁴²

The highest amount of cobalt (21.07) was found from **T₃** and the lowest amount of cobalt (9.30) was counted under **T₄** (Table 2). The highest percentage of molybdenum (13.10) was counted under **T₁** and for the lowest percentage of molybdenum (4.27) was counted under **T₆** (Table 2). Percentage of selenium was counted highest under **T₅** (9.10) and lowest percentage of selenium was counted under **T₁** (1.77) as shown in table 2.

Table 2. Effect of sawdust substrates on mineral contents of *Pleurotus florida*.

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (%) mg	Zn (%) mg	Co (%) mg	Mo (%) mg	Se (%) mg
T ₁	4.29ab	0.89a	1.17c	30.38ab	14.70bc	41.57b	27.97ab	12.10bc	13.10a	1.77e
T ₂	4.38a	0.86ab	1.24bc	27.66b	13.68c	42.28b	27.86b	13.90bc	9.76ab	7.27ab
T ₃	4.09c	0.83b	1.26bc	29.40ab	16.99ab	40.71b	30.26a	21.07a	8.74b	2.90de
T ₄	4.43a	0.84ab	1.39a	32.53ab	19.96a	44.69a	21.05bc	9.30c	8.98b	4.83cd
T ₅	4.17b	0.85b	1.28b	33.35a	14.68bc	40.77b	18.70c	9.50c	4.29c	9.10a
T ₆	4.11b	0.88a	1.17c	32.59ab	16.31bc	41.07b	21.12b	16.27ab	4.27c	5.30bc
CV (%)	5.32%	3.64%	4.03%	9.94%	5.92%	2.52%	9.29%	10.62%	11.78%	23.03%
LSD _(0.05)	0.41	0.06	0.10	5.60	3.03	1.92	4.14	2.38	2.18	2.18

Means followed by same letter significantly different at 1% or 5% level of significance.

CONCLUSION

Effect of various sawdust substrates on the nutritional composition of *Pleurotus florida* was analyzed. Amongst all the treatment the highest phosphorous was obtained from **T₁** substrate. The highest amount of lipid, protein, ash, moisture and molybdenum were obtained from **T₂** sawdust substrate. The highest amount of cobalt and zinc were obtained from **T₃** sawdust substrate. The highest amounts of carbohydrate, dry matter, nitrogen, potassium, magnesium and iron were obtained from **T₄** sawdust substrate. The highest amount of crude fiber, calcium and selenium were obtained from **T₅** substrate. The lowest amount of lipid was found from **T₆** substrate. Therefore observing the nutritional composition it is apparent that **T₄** substrate is the best amongst all the applied treatment for locally producing popular *Pleurotus florida* mushroom variety in Bangladesh. In pharmaceutical aspects,

Pleurotus florida mushroom has been proven to be highly significant in the context of our country's economy and providing nutrition to the people as well. As the inclusion of whole mushrooms into the diet may have efficacy as potential dietary supplements.

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