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Recycling of Spent Mushroom Substrate for the Production of Oyster Mushroom

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The huge amount of spent mushroom substrate (SMS) was disposed, especially around the mushroom cultivation complexes. The present study aimed at utilizing this SMS in a productive and sustainable way. Experiment was carried out to reuse of SMS of oyster mushroom for the production of oyster mushroom at Bangladesh Agricultural University (BAU), Mymensingh. Two mushroom species (*Pleurotus ostreatus* and *P. florida*) were grown on SMS supplemented with sawdust and wheat bran at different proportions. The results showed that SMS supplement with 60% sawdust + 20% wheat bran demonstrated the highest biological yield, economic yield and biological efficiency for both *P. ostreatus* and *P. florida.* Yield parameters were increased with increasing C/N ratio where as 36:1 C/N ratio exhibited the highest yield. The C/N ratio below or above 36:1 decreased yield of both species of oyster mushroom. The optimum C/N ratio for economic yield varied between the two oyster mushroom species and found to be 35.2 for *P. ostreatus* against C/N ratio of 40.1 for *P. florida*. Concerning biological yield and biological efficiency the optimum C/N ratio was found 35.7 for *P. Ostreatus* and 40.6 for *P. florida*. The study emphatically indicated that reuse of spent mushroom substrate with supplementation can be a good solution to address the disposal problem where as supplemented SMS can be a good substrate for further mushroom production.

Keywords: Spent mushroom substrate, Oyster mushroom, Yield, Biological efficiency, Carbon nitrogen ratio

Mushroom, the fruiting body of macrofungi, is an important food item with its high nutritive and medicinal values. Mushroom cultivation in Bangladesh has consistently been increasing and producing huge amount of mushroom waste that is spent mushroom substrate every year. About five kilograms of fresh compost are needed to produce one kilogram of mushrooms (Sample et al., 2001). There was an estimate that about 70,000,000 metric tons of mushroom waste was generated during the year 2007 in the world (Tajbakhsh et al., 2008). Across the country (Bangladesh) per day about 60,000-70,000 of spawn packets were prepared to produce 15-20 MT of fresh mushrooms in the year 2008 (Amin, 2008). As an average 0.5 ton mushroom waste is produced from each ton of spawned compost (Levanon et al., 1994), a huge amount of SMS is added to the burden of the municipal refuses, especially around the mushroom cultivation complexes.

The environmental degradation caused by inadequate disposal of spent mushroom waste can be expressed by the contamination of surface and ground water through leachate, soil contamination through direct waste contact or leachate, air pollution by burning of wastes, spreading of diseases by different vectors like birds, insects and rodents, or uncontrolled release of methane by anaerobic decomposition of waste (Visvanathan and Glawe, 2006). Hence, there exists a good opportunity to use spent mushroom substrate in a productive way.

The status of waste management in Bangladesh is far from satisfactory. Problems arising from solid waste are increasing drastically. Methods of waste disposal have not been in Bangladesh (BCAS, 2001). Until recently, mushroom waste as an agricultural waste has been disposed of without due consideration to the environment. Mushroom waste is disposed of locally, either by land spreading or by landfill. Preventing waste gets top priority to manage waste in sustainable way. When waste is created, it gives priority to preparing it for re-use, then recycling, then recovery, and last of all disposal (e.g. landfill) (Defra, 2011). A a huge amount of SMS is producing daily from mushroom farm, reuse or recycling may be an effective way to manage this solid waste. Spent mushroom substrate does not produce good yield when it is used again in mushroom production as a substrate, because gradual depletion of nutrients occurs due to their subsequent utilization of mushroom mycelium (Sharma and Jandaik, 1985). Beyer (2007) also reported that the practice of recycling spent mushroom substrate in mushroom production is not economically feasible because it adds little nutritional value. On the other hand mushrooms depend on substrates for nutrition. The nature and the nutrient composition of the substrate affect mushroom quality and crop yield of biotransformation process (Philippoussis et al., 2001; Philippoussis et al., 2003). Mycelium growth and mushroom production both are affected by cellulose, hemicelluloses and lignin proportions along with nitrogen content of the cultivation substrate (Mata and Savoie, 2005).

Nutritional supplementation of cultivation substrate is an important cultural practice of mushroom cultivation. Substrate supplementation with protein rich materials was proved to enhance yield of *Agaricus, Pleurotus and Lentinulastrains.* (Naraian et al., 2008). Yoshida et al. (1993) reported the highest yield with substrates (chopped straw or sawdust) mixed with wheat bran, rice bran and bean curd refuse at 45%. In this way nutritional supplementation of SMS may be a solution to enrich spent mushroom substrate to produce good yield of mushroom. In Bangladesh, little work is done on the performance of different species of oyster mushroom grown on the agricultural byproducts, wastes, grasses as substrates. There is very little evidence on the use of mushroom waste to grow mushrooms again. There is ample scope of using spent mushroom substrate for further utilization after appropriate supplementation. Therefore, the goal of the present study was to examine whether the spent mushroom substrate of oyster mushroom with supplement can be used to produce oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus florida)*.

Materials and methods

The experiment was carried out in the Laboratory of the Department of Soil Science of Bangladesh Agricultural University (BAU), Mymensingh. Spent Mushroom substrate was collected from different mushroom farms located at different places of Mymensingh Sadar Upazila and from Mushroom Palli located at Savar, Dhaka of Bangladesh.

Selection of species

Two species of oyster mushroom *(Pleurotus ostreatus* and *Pleurotus florida*) were employed to see the effects of spent mushroom substrate (SMS) as a substrate material for mushroom production. These two species are commonly grown in Bangladesh.

Treatments

Sawdust and wheat bran at different proportions were mixed with SMS as the supplements. The experiment consisted of 12 treatments including control, as follows.

T_1: SMS
T_2: SMS + Sawdust (70:30)
T_3: SMS + Sawdust (60:40)
T_4: SMS + Sawdust (50:50)
T_5: SMS + Sawdust (40:60)
T_6: SMS + Sawdust (20:80)
T_7: Sawdust + Wheat bran (65:35)
T_8: SMS + Sawdust + Wheat bran (20:60:20)
T_9: SMS + Sawdust + Wheat bran (30:50:20)
T_{10}: SMS + Sawdust + Wheat bran (40:40:20)
T_{11}: SMS + Sawdust + Wheat bran (50:30:20)
T_{12}: SMS + Sawdust + Wheat bran (60:20:20)

A control substrate was made with SMS only (treatment T_1). Again another substrate was made with sawdust and wheat bran without spent mushroom substrate at a ratio of 65:35 (treatment T_7) which is usually used by the farmers to produce mushroom in Bangladesh. The experiment was laid out in a completely randomized design (CRD) with three replications of each treatment. Organic carbon and nitrogen contents of spent mushroom substrate and other supplements with sawdust and wheat bran were determined. C/N ratio of different treatments were calculated from the values of organic carbon and nitrogen content.

Preparation of spent mushroom substrate

Spent mushroom substrate was used as the base material for further growing of mushroom. Different nutritive materials such as sawdust and wheat bran were used as the supplements. Collected spent mushroom substrate of *P. ostreatus* was passed through a mechanical shredder to transform into granular form and then spread on a concrete floor in a covered building and allowed to air dry for 3-4 days to reduce the moisture content at approximately 45%. Air dry spent mushroom substrate was mixed with supplemented materials at a ratio shown against different treatments.

Cultivation of oyster mushroom

Spent mushroom substrate of *Pleurotus ostreatus* was used alone or in combination with sawdust and wheat bran as a substrate to grow *Pleurotus ostreatus* and *Pleurotus florida.* Five hundred gram of prepared materials were poured into heat resistant polypropylene bags of 10 inch x 7 inch size. The bags were inoculated with the mother cultures of two oyster species after autoclaving and cooling. The packets were incubated in a cool (25 ± 2°C temperature) and dark place for 20-25 days till the packets became full with white mycelia. After completion of spawn running, the polythene bags were removed from the air conditioned room and the packets were kept on a rack in the culture house. Four to five harvests were done during the total growing period. Data were recorded on the stimulation to primordia initiation and primordia initiation to the 1st harvest period and also on the yield of oyster mushroom. This was done three times, each period was of four months starting from January to December.

Calculation of the relationship of yield parameter with substrate C/N ratio

The relationship between yield parameters of oyster mushroom with C/N ratio was examined by correlation statistics (Fig. 1 & Fig. 2) to find out the optimum C/N ratio for achieving higher mushroom yield as well as higher biological efficiency. For it, a response curve was drawn by fitting the values of mushroom yield or biological efficiency to the 'y' axis and that of C/N ratios to the 'x' axis, following quadratic equation, $y= a + bx + cx^2$. The optimum C/N ratio was computed for obtaining the maximum biological yield, economic yield and biological efficiency from the regression equations following the procedure as outlined by Gomez and Gomez (1984).

Statistical analysis

Data were statistically analyzed by MStat-C programme. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% level (Gomez and Gomez, 1984).

Results

The experiment was carried out to study the suitability of spent mushroom substrate (SMS) for the production of oyster mushroom (*Pleurotus ostreatus* and *Pleurotus florida)* with supplement.

Stimulation to primordia initiation, primordia initiation to 1st harvest and effective fruiting bodies of oyster mushroom: Addition of sawdust or the mixture of sawdust and wheat bran did not influenced significantly on the number of days required from stimulation to primordia initiation and primordia initiation to the 1st harvest of oyster mushroom (*P. ostreatus* and *P. florida*) (Table 1).

Table 1: Effects of different treatments on Stimulation to primordia initiation, Primordia initiation to 1st harvest and no. of effective fruiting bodies of two species of oyster mushroom (*Pleurotus ostreatus* & *Pleurotus florida)*

Treatments	*Pleurotus ostreatus*			*Pleurotus florida*		
	SPI (Days)	PIFH (Days)	No. of EF (no. packet^{-1})	SPI (Days)	PIFH (Days)	No. of EF (no. packet^{-1})
T_1 : SMS	5.00	3.44	29. 9 f	5.22	3.78	12.6 e
T_2: SMS + SD (70:30)	3.67	4.00	32.1 de	4.78	3.89	14.2 cd
T_3 : SMS + SD (60:40)	3.67	4.00	33.4 bc	4.22	3.56	14.7 cd
T_4 : SMS + SD (50:50)	3.33	3.89	35.6 a	4.33	4.11	16.4 b
T_5: SMS + SD (40:60)	3.33	3.56	36.2 a	4.22	3.33	17.7 a
T_6: SMS + SD (20:80)	4.33	3.44	31.9 e	5.00	3.44	14.6 cd
T_7: SD + WB (65:35)	4.00	4.22	31.7 e	4.67	4.22	16.7 ab
T_8: SMS+SD+WB (20:60:20)	3.33	4.00	34.3 b	4.44	3.89	15.3 c
T_9: SMS+SD+WB (30:50:20)	3.67	3.44	32.7 c-e	4.44	3.78	14.6 cd
T_{10}:SMS+SD+WB (40:40:20)	3.67	4.00	33.4 bc	4.33	3.33	14.3 cd
T_{11}: SMS+SD+WB (50:30:20)	3.67	3.67	33.2 b-d	4.67	3.78	13.8 d
T_{12}: SMS+SD+WB (60:20:20)	4.00	3.67	33.3 bc	4.78	3.67	12.6 e
CV (%)	15.71	12.24	2.06	10.34	13.40	4.03
Level of significance	NS	NS	**	NS	NS	**
SE (±)	0.33	0.27	0.40	0.26	0.29	0.34

Values having same letters in a column do not differ significantly at 5% level by DMRT. NS=Not significant, ** = Significant at 1% level, CV = Coefficient of variation, SMS = Spent mushroom substrate, SD = Sawdust, WB=Wheat bran, SPI = Stimulation to primordia initiation, PIFH = Primordia initiation to 1st harvest, EF = Effective fruiting bodies

But the number of effective fruiting bodies of both mushroom species varied significantly due to different SMS treatments. Spent mushroom substrate supplemented with sawdust with or without wheat bran increased effective fruiting bodies up to 60% SD supplement then it decreased. All the sawdust and sawdust + wheat bran supplemented treatments (T_1 to T_{12}) increased the number of effective fruiting bodies compared to control (T_1) treatment except treatment T_{12} in *P. florida*. Compared to T_7 (farmer's practice), all the treatments except T_1, T_6

and T_9 demonstrated higher yield in *P. ostreatus* and T_5 in *P. Florida*.

Biological and economic yield of oyster mushroom: Biological and economic yield of oyster mushroom grown on SMS with different proportions of sawdust varied significantly. Increased yield (biological & economic) was observed in all treatments supplemented by sawdust (T_1 to T_6) at different proportions (Table 2). Both yield was increased with the increasing rate of sawdust up to SMS: sawdust, 40:60 (T_5) and thereafter it decreased (T_6). In case of supplementation with the mixture of sawdust and wheat bran (from T_7 to T_{12}), the highest yield was found in treatment T_8 and further increase in wheat bran decreased the biological yield of mushroom.

Comparing the treatments supplemented with sawdust and sawdust + wheat bran, the highest yield (biological & economic) was obtained in all supplemented treatment compared to T_1 (SMS). *P. ostreatus* produced higher biological yield in treatments T_8, T_5, T_4, T_9 & T_{10} and *P. florida* gave the highest biological yield in treatments T_8 & T_5 over farmer's practice (T_7).

Biological efficiency of oyster mushroom: The biological efficiency of *P. ostreatus* mushroom production varied significantly with different combinations of sawdust and sawdust + wheat bran supplements. Treatment T_8 recorded the highest biological efficiency which was 107.5% followed by T_5 (105.1%) treatments. The lowest biological efficiency was observed in treatment T_1 (78.4%) (Table 2). Biological efficiency of *P. florida* was recorded highest in treatment T_8 (92.7%) followed by treatment T_5 (88.9%). The lowest biological efficiency of *P. florida* was recorded in treatment T_1 (66.8).

Table 2: Effects of different treatments on biological yield, biological efficiency and economic yield of two species of oyster mushroom (*Pleurotus ostreatus* & *Pleurotus florida)*

Treatments	*Pleurotus ostreatus*			*Pleurotus florida*		
	BY (g packet^{-1})	BE (%)	EY (g packet^{-1})	BY (g packet^{-1})	BE (%)	EY (g packet^{-1})
T_1 : SMS	141 j	078.4 j	139 j	120 k	66.8 k	117 j
T_2: SMS + SD (70:30)	151 i	084.2 i	146 i	132 i	73.6 i	129 h
T_3 : SMS + SD (60:40)	154 h	085.5 h	149 h	138 h	76.9 h	135 g
T_4 : SMS + SD (50:50)	186 c	103.6 c	182 c	146 f	81.3 f	143 e
T_5: SMS + SD (40:60)	189 b	105.1 b	185 b	160 b	88.9 b	156 b
T_6: SMS + SD (20:80)	167 f	092.8 f	156 g	155 d	86.3 d	151 c
T_7: SD + WB (65:35)	170 e	094.8 e	166 f	157 c	87.5 c	153 c
T_8: SMS+SD+WB (20:60:20)	193 a	107.5 a	195 a	167 a	92.7 a	164 a
T_9: SMS+SD+WB (30:50:20)	178 d	099.0 d	174 d	149 e	82.9 e	146 d
T_{10}: SMS+SD+WB(40:40:20)	176 d	098.1 d	172 e	143 g	79.3 g	140 f
T_{11}: SMS+SD+WB (50:30:20)	162 g	090.2 g	156 g	132 i	73.5 i	130 h
T_{12}: SMS+SD+WB (60:20:20)	154 hi	085.3 hi	149 h	126 j	70.0 j	122 i
CV (%)	0.76	0.76	0.48	0.84	0.84	0.81
Level of significance	**	**	**	**	**	**
SE (±)	0.74	0.41	0.45	0.69	0.39	0.66

Values having same letters in a column do not differ significantly at 5% level by DMRT. NS=Not significant, **= Significant at 1% level, CV = Coefficient of variation, SMS = Spent mushroom substrate, SD = Sawdust, WB=Wheat bran, BY=Biological yield, EY= Economic yield, BE= Biological efficiency

Sawdust supplementation with SMS increased biological efficiency of mushroom production up to a level then decreased. Among treatments (T_1 to T_6), biological efficiency was increased with increasing proportion of sawdust up to treatment T_5 and after that a decreasing trend was noticed. When the SMS was supplemented with sawdust + wheat bran (T_7 to T_{12}), the highest biological efficiency was observed in treatment T_8. The values were decreased with decreasing amount of sawdust up to treatment T_{12}.

All the treatments receiving SMS with sawdust and/or sawdust + wheat bran, higher biological efficiency of mushroom was observed over SMS treatment (T_1). The treatments of *P. ostreatus* T_8, T_5, T_4, T_9 and T_{10} showed higher biological efficiency over farmers' practice (T_7) and in case of *P. florida*, T_8 and T_5 treatments had higher biological efficiency over farmers' practice (T_7).

Relationship of yield parameters with substrate C/N ratio: Organic carbon and nitrogen levels of substrate are important for mushroom culture and are expected to influence mushroom yields. Indeed, their ratio (C/N ratio) is very important in consideration of their role on mushroom growth.

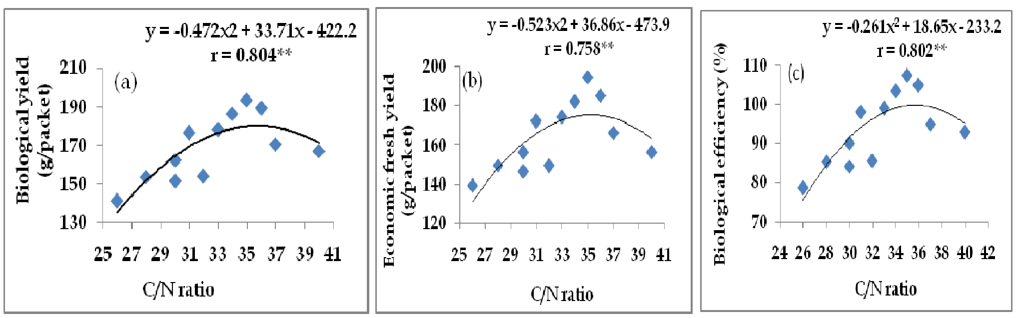

Figure 1: Relationship between C/N ratio level and (a) Biological yield, (b) Economic yield, (c) Biological efficiency of oyster mushroom (*P. ostreatus*)

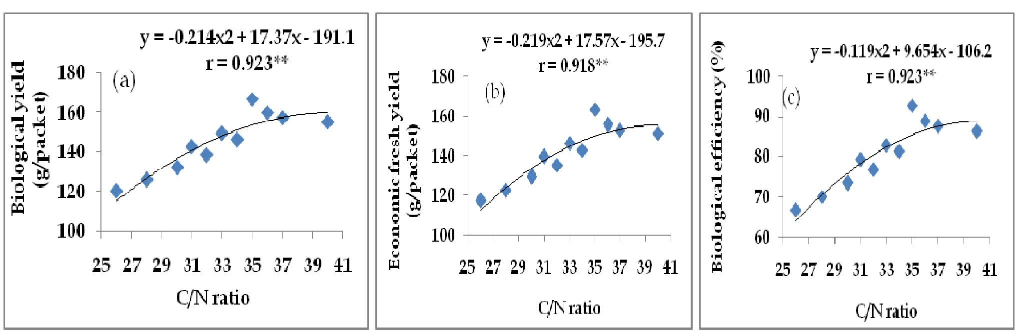

Figure 2: Relationship between C/N ratio level and (a) Biological yield, (b) Economic yield, (c) Biological efficiency of oyster mushroom (*P. florida*)

The optimum C/N ratio for obtaining the maximum biological yield, economic yield and biological efficiency are calculated from the regression equations as shown in Table 3. It appears that the optimum C/N ratio varied between the two oyster mushroom species. The optimum C/N ratio for obtaining economic yield was found to be 35.2 for *P. ostreatus* against C/N ratio 40.1 for *P. florida*. Concerning biological yield and biological efficiency the optimum C/N ratio was found 35.7 for *P. Ostreatus* and 40.6 for *P. florida*.

Table 3: Optimum C/N ratio for biological yield, economic yield and biological efficiency of *P. ostreatus* and *P. florida*

Variables	*P. ostreatus*		*P. florida*	
	Equations	Opt. C/N ratio	Equations	Opt. C/N ratio
Biological yield vs C/N ratio	$Y= -0.472x^2+33.71x -422.2$	35.7	$Y= -0.214x^2 +17.37x -191.1$	40.6
Eonomic yield vs C/N ratio	$Y=-0.523x^2+36.86x - 473.9$	35.2	$Y= -0.219x^2+17.57x - 195.7$	40.1
Biological efficiency vs C/N ratio	$Y= -0.261x^2+18.65x - 233.2$	35.7	$Y= -0.119x^2 + 9.654x - 106.2$	40.6

Discussion

The present study demonstrates that SMS can be used as a potential substrate for mushroom production and supplementation of SMS with sawdust and wheat bran can give even higher yields. In case of *P. ostreatus*, 50% or 60% sawdust supplement and 40%, 50% or 60% sawdust with 20% wheat bran supplement and in case *P. florida* 60% sawdust with 20% wheat bran supplement produced higher biological yield, economic yield and biological efficiency not only over non-supplemented SMS substrate but also over farmers' practice. SMS supplemented by 60% sawdust with 20% wheat bran was found more effective. Yoshida et al. (1993) reported the highest yield with substrate mixed with wheat bran, rice bran and bean curd refuse at 45% supplement.

Depletion of nutrients occurred in the substrate due to subsequent utilization of nutrients by mushroom mycelium. For this reason, SMS alone was not able to produce good yield of mushroom. The sawdust and wheat bran added SMS produced higher yields compared to sole SMS. Siddhant and Singh (2009) utilized spent mushroom substrate as the ingredient in cultivation of three *Pleurotus* spp. (*P. sajor-caju*, Strai-Malaysia, *P. florida*, Strain P1 and *P. Flabellatus*) and recorded the highest yield and biological efficiency in the sets supplemented with 25% proportions of SMS. *Pleurotu*s waste also showed significantly higher yield of *P. sajor-caju* on starch, peptone and wheat bran supplemented spent mushroom substrate (Sharma and Jandaik, 1985). Shashirekha et al. (2005) also noted increased biomass and mushroom's productivity due to supplementation.

Physical and chemical properties of substrates have a great effect on the yield and biological efficiency of *Pleurotus* spp (Ozcelik and Peksen, 2007; Peksen and Yakupoglu, 2009). Composition of substrate has also a great influence on the yield and biological efficiency (Fanadzo et al., 2010). A high fiber content and C/N ratio could enhance the digestibility of the lingo-cellulose content, followed by high availability of cellulose materials as mushroom nutrients. In this issue, nitrogen might be in the bound form which needs more time to be delivered to the mushroom's mycelia (Fandzo et al., 2010). In the present study, sawdust which is a rich source of carbon was used as supplement. With the increase of sawdust and wheat bran supplementation, the total amount of carbon and nitrogen in the substrate were also increased which thus affected the C/N ratio of the substrate.

Increasing C/N ratio in the present study affected positively the biological and economical yields of oyster mushroom. The highest yield was recorded in treatment containing 36:1 C/N ratio and thereafter the yield was decreased. The optimum C/N ratios for obtaining the maximum biological yield, economic yield and biological efficiency are calculated from the regression equations. The optimum C/N ratio varied between the two oyster mushroom species. The optimum C/N ratio for obtaining economic yield was found to be 35.2 for *P. ostreatus* against C/N ratio 40.1 for *P. florida*. Sarkar et al. (2008) who found that carbon content in the substrate had an increasing effect on economic yield to oyster mushroom up to a level and further increase in the carbon content in the substrate had decreasing effect on economic yield and C/N ratio of 36:1 supported the best growth of mushroom. Philippoussis et al. (2000) also reported a positive correlation between the C/N ratio and *Pleurotus eryngii* mushroom yield. They also demonstrated that mycelium growth rates of *Pleurotus* spp., *L. edodes* were positively correlated to C/N ratio (Philippoussis et al., 2001, 2003).

Kues and Liu (2000) determined the optimal C/N ratio for fruiting of *A. bisporus* to lie between 80:1 and 10:1. For fruiting body induction, it is important to keep a balance between C and N source (Kues and Liu, 2000; Stamets, 2000). A well balance carbon to nitrogen ratio enhances the growth and development of mushroom while an imbalance of C/N impedes their growth (Okhuoya et al., 2000). Spent mushroom substrate supplemented with sawdust and wheat bran at different proportions can be used for mushroom production again.

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