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Integrated Use of Low Cost Materials with Traditionally Used Sawdust in Oyster Mushroom (*Pleurotus Ostreatus*) Cultivation in Sri Lanka

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Abstract

Although several alternative substrates have been introduced, Sri Lankan Oyster mushroom (*Pleurotus ostreatus*) farmers rely only on sawdust. Currently, there is difficulty in finding traditionally used sawdust as they are used in other industries. Therefore, a study was conducted to cut off the traditionally used mango sawdust (MSD) requirement by combining low-cost natural materials such as Guinea grass (*Panicum maxima*) leaves (GL) and paddy husk (PH), and another potential sawdust - teak sawdust (TSD). The experiment was designed as a Complete Randomized Design (CRD) with eight substrates; T1: 100% MSD (Control), T2: 50% MSD + 50% PH, T3: 25% MSD + 75% PH, T4: 50% MSD + 50% GL, T5: 25% MSD + 75% GL, T6: 100% TSD, T7: 75% MSD + 25% TSD, T8: 50% MSD + 50% TSD. All the treatments comprised the substrate (30 kg), rice bran (3 kg), mungbean flour (300 g), Calcium oxide (600 g), and Magnesium Sulphate (60 g). Among the substrates evaluated, T1 showed the highest mycelium growth rate (6.83 mm/day), while T7 showed the lowest (5.78 mm/day); T3 spent a minimum duration for primordia initiation (07 days) and first harvest (10 days from the bag opening and 39 days from the inoculation); T7 showed the highest cap diameter (8.07 cm) which is statistically similar to all other treatments except in T3 (7.47 cm); and T4 showed the highest dry weight (46.18 g/kg) and the fresh weight (561.07 g/kg) of harvested mushroom. The biological efficiency was significantly higher in T4, T5 and T2, and it was significantly lower in T7, T6 and T8 than the control. Therefore, incorporation of guinea leaves or paddy husk would be effective to reduce the amount of traditionally used mango sawdust in Oyster mushroom cultivation. Further, Teak sawdust would not as effective as traditionally used mango sawdust.

***Key words**: Teak sawdust, Panicum maxima, paddy husk, biological efficiency*

Introduction

Since time immemorial, many people have consumed wild mushroom as a food source. Initially, these edible wild mushrooms were gathered by hunting, but later people began to cultivate mushrooms because they were not available naturally throughout the year (Gamage and Ohga, 2018). At present, mushroom cultivation has become popular as a reliable source of food and a sustainable source of income, particularly among rural communities in the developing world (Higgins et al., 2017). Mushroom cultivation is also an environmentally benign, profitable agri-business that converts low-cost lingo-cellulosic material into protein-rich foods without requiring high capital investments and maintenance (Marshall and Nair, 2009; Rathod et al., 2021). Mushroom is also called as 'white vegetables' or 'boneless vegetarian meat' due to its high protein, vitamin, and fiber contents (Abirami et al. 2023). In addition, mushroom possess certain medicinal properties bioactivities (Amin and Ganapathy, 2023).

In Sri Lanka, mushrooms have contributed to the well-being of local livelihoods since the introduction of commercial mushroom cultivation in 1985 (Karunarathna et al., 2017). Currently, there are four main mushroom varieties being cultivated in Sri Lanka namely American oyster (*Pleurotus ostreatus*), Abalone (*P. cystidiosus*), Bhutan oyster (*P. eous*), and Button mushroom (*Agaricus bisporus*) (Fernando et al., 2022). Of these varieties, *P. ostreatus* is the most popular among the Sri Lankan mushroom growers (Karunarathna et al., 2017). All the mushroom farmers in Sri Lanka rely on sawdust as the substrate for *P. ostreatus* cultivation due to the convenience and year-round availability (Thilakarathne and Sivasankar, 2018). It has been found that sawdust of soft-textured woods such as Mango, Lunumidella, Mahogany and Rubber ideal for cultivation of *P. ostreatus* (Hami, 1990; Joseph et al., 1998).

However, at present mushroom growers are in a difficulty to find sawdust, as many of sawdust suppliers have started to make furniture from sawdust, which are often used as mushroom substrates (Fernando et al., 2022). Therefore, it is timely to investigate alternative cultivation media to sustain mushroom production in Sri Lanka. Rajapakse et al., (2007) introduced paddy straw and sugarcane bagasse as alternative mushroom substrates, which proved to be equally good as sawdust for *P. ostreatus*. However, mushroom growers in Sri Lanka still lack a good perception on alternative substrates rather than relying on sawdust (Thilakarathne and Sivasankar, 2018). In the present study, we attempt to introduce an integrated approach to mushroom substrates by blending locally available low-cost materials with traditionally used sawdust instead of completely replacing the traditionally used sawdust.

We evaluate the performance of different combinations of three substrate materials namely teak sawdust that is not a soft-wood that mushroom farmers traditionally use; guinea grass (*Mana* or *Panicum maxima*), a perennial invasive weed in Sri Lanka that grows naturally in all ecological zones and invades forest gaps, roadsides, grasslands and agricultural lands; and rice husks, a waste product generated during the rice milling process with the traditionally used mango sawdust for *P. ostreatus* cultivation.

Methodology

Location

The study was conducted at the mushroom unit, faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura.

Experimental design and treatment combinations

The experiment was planned as a Complete Randomize design (CRD) with triplicates. There were eight treatments each comprised with 30 kg of substrate, 3 kg of rice bran, 300 g of mungbean flour, 600 g of Calcium Oxide (CaO), and 60 g of Magnesium Sulphate (MgSO4) (DOA, 2022). In each treatment, there were different substrate compositions of mango sawdust , teak sawdust , guinea grass and paddy husk as follows: T1: 100% mango sawdust, T2: 50% mango sawdust + 50% paddy husk, T3: 25% mango sawdust + 75% paddy husk, T4: 50% mango sawdust + 50% guinea leaves, T5: 25% mango sawdust + 75% guinea leaves, T6: 100% teak sawdust, T7: 75% mango sawdust + 25% teak sawdust, T8: 50% mango sawdust + 50% teak sawdust. Each treatment was triplicated.

Preparation of mushroom growing media

Guinea grass were harvested before flowering, chopped into 1-2 cm size, and sun dried to remove excess moisture. Before media preparation, dried Guinea grass and paddy husks were treated with 2% Calcium Hydroxide [$Ca(OH)_2$] solution for 24 hours in order to soften the cell walls for easy penetration of fungal mycelia. Substrate materials were dried before preparation of mushroom growing media. All the ingredients were mixed thoroughly by adding water and moisture content checked by palm squeezing method (Boulware et al., 2014). The prepared mushroom growing media were filled into 7" width and 14" height gauge 300 polypropylene bags gently pressing by hand. A ¾" width and ½" height PVC ring was inserted into the collar of bag, sealed using cotton plug and covered with a paper piece. The prepared bags were steam sterilized under 100 ^{0}C and high pressure for 3 hours.

Cultivation of Oyster mushroom

Oyster mushroom (*P. ostreatus*) spawns were obtained from seed and planting material center Kundasale, Sri Lanka. First, the sterilized bags were allowed to cool before inoculation of mushroom spawns. The paper piece and cotton plug were then removed and a table spoonful of mushroom spawn was inoculated into the bags containing growing media under aseptic conditions. Then the inoculated bags were sealed with a sterile cotton plug and covered with a sterile paper piece. The bags were then incubated for 3-4 weeks under dark conditions with uncontrolled temperature and RH. The incubated bags were then transferred into the cropping room after full colonization of mycelium. The bags were opened for cropping by removing the necks and keeping them under 32ºC temperature and 86% RH. Mushrooms were harvested manually when they got matured by twisting to uproot from the base.

Data collection and measurements

C:N ratio of substrates

The C:N ratio was determined after estimating the total nitrogen and total carbon content of each substrate following kjeldahl oxidation method (Anderson and Ingram, 1989) and loss-on-ignition method (Goldin, 1987), respectively.

Mycelium growth rate

The growth rate of fungal mycelium was measured during the incubation by taking linear length of the mycelium (beginning from the neck of the bag) at three different places of the bag, periodically. The first measurement was taken 7th day after inoculation and then it was regularly taken every 4-day interval up to full colonization. Mycelium growth rate was determined by the following formula: Average length of mycelium (mm)/ number of days (Sarker, 2004).

Time taking to primordia initiation and first harvesting

The number of days were counted from the bay opening to primordia initiation and first harvesting, respectively.

Mushroom cap diameter (cm)

Broadness of cap was determined by measuring average diameter of the cap using randomly selected 15 mushrooms per each treatment after harvesting.

Fresh and dry weights of mushroom

The harvested mushroom was weighed on an electronic balance (Model: Kern EMB 6000-1, Germany) to get the fresh weight. The dry weight was measured after drying of mushroom in an oven (Model: 3511-11, China) at 60ºC temperature for 48 hours. Both fresh and dry weights were determined with respect to 1kg of each substrate.

Biological efficiency

The biological yield (g/kg) was determined by measuring the weight of all cluster of fruiting bodies produced during the cropping period and dividing it by initial weight of the substrate (Li et al., 2017).

Data analysis

One-way analysis of variance (ANOVA) was conducted with Duncan's multiple range tests to compare the mean significant differences ($p < 0.05$) among each treatments by using computer software on SAS version 9.0.

Results and discussion

Mycelium growth on different substrates

Daily mycelium growth of *P. ostreatus* on different substrates showed in Table 1. Mycelial expansion serves as an initial phase that establishes favorable internal circumstances for the formation of fruiting bodies. Consequently, robust mycelial growth plays a crucial role in the successful cultivation of mushrooms (Pokhrel et al. 2009). The highest mycelium growth rate was observed in sole mango sawdust (T1) treatment (6.83 mm/day) while the lowest was observed in 75% mango sawdust + 25% teak sawdust (T7) treatment (5.78 mm/day). Sole teak sawdust (T6) and supplementation of the mango sawdust using teak sawdust (T7 and T8) showed a significantly decline in the mycelium growth rate compared to the supplementation of the mango sawdust with paddy husk and guinea leaves (T2-T5). This difference could be due to the high C: N ratios (Table 1) and the compacted nature of teak sawdust-added treatments. Chukwurah et al. (2012) has suggested that the lack of proteinaceous materials in the substrates and/or the physical properties of the materials used are the reasons behind the poor mycelium growth rate of oyster mushroom during the incubation period. Li et al. (2017) also reported that the nitrogen deficiency is known to inhibit the hyphal growth, hence the substrates containing high C:N ratio resulted slow spawn running rates. Further, it is considered that the antifungal properties present in the teak sawdust (Asdaq et al., 2022) may inhibit the fungal colonization within the substrate. Similar to the present results, Baysal et al. (2003) and Thongklang and Luangharn (2016) also obtained accelerated mycelium growth rates in paddy husks added treatments. Incorporation of paddy husk and guinea leaves with the mango sawdust enhanced the porosity which led more aeration within the substrate that eventually affected on the proliferated mycelium growth.

Table 1. C:N ratio, mean mycelium growth rate, Number of days required to primordia initiation and first harvest in different substrates

T	C:N ratio	Mean mycelium growth (mm/day) (±SD)	Days required to primordia initiation (±SD)	Days from bag opening to 1st harvest (±SD)	Days from inoculation to 1st harvest (±SD)
T1	286.39	6.83 ± 0.07^{a}	14 ± 1.15^{b}	19 ± 0.67^{ab}	44 ± 0.67^{b}
T2	246.96	6.51 ± 0.02^{b}	11 ± 1.53^{c}	15 ± 1.53^{c}	43 ± 0.88^{b}
T3	192.71	6.72 ± 0.04^{ab}	7 ± 00^{d}	10 ± 00^{d}	39 ± 00^{d}
T4	191.20	6.52 ± 0.01^{b}	8 ± 00^{d}	12 ± 0.33^{d}	41 ± 0.33^{c}
T5	187.78	6.72 ± 0.10^{ab}	8 ± 0.58^{d}	11 ± 0.88^{d}	40 ± 0.88^{cd}
T6	572.80	6.09 ± 0.12^{c}	16 ± 1.53^{a}	20 ± 1.16^{ab}	44 ± 0.66^{b}
T7	455.23	5.78 ± 0.02^{d}	14 ± 0.58^{b}	17 ± 0.33^{b}	43 ± 0.33^{b}
T8	427.16	6.00 ± 0.16^{cd}	16 ± 0.58^{a}	21 ± 0.33^{a}	46 ± 0.33^{a}

T: Treatment; (T1: 100% mango sawdust, T2: 50% mango sawdust + 50% paddy husk, T3: 25% mango sawdust + 75% paddy husk, T4: 50% mango sawdust + 50% guinea leaves, T5: 25% mango sawdust + 75% guinea leaves, T6: 100% teak sawdust, T7: 75% mango sawdust + 25% teak sawdust, T8: 50% mango sawdust + 50% teak sawdust; Means with different letters are statistically different at $p<0.05$)

Time (days) taking to primordia initiation and first harvesting

The time duration required for primordia initiation and first harvesting in different substrates were presented in table 1. T3 treatment (25% mango sawdust + 75% paddy husk) showed the fastest growth rate spending a minimum number of days for the primordia initiation (07 days), first harvest from the bag opening (10 days) and first harvest from the inoculation (39 days). Overall, the teak sawdust incorporated substrates and sole mango sawdust required the highest time period for the primordia initiation (14-16 days) and first harvest (17-21 days from the bag opening and 43-46 days from inoculation).

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The result of the current study is in line with Vetayasuporn's (2006) study that primordia were formed 2-3 weeks after the completion of the mycelium growth. Further, Vetayasuporn (2007) suggested that P. ostreatus began to appear in their primordia within 10-14 days after the opening of the cylindrical bags and Bhattacharjya et al. (2014) stated that it was ranged from 6.0-8.0 days. However, the present results are not tally with Ruiz-Rodriguez et al. (2010) findings; where they found that pin heads of *P. ostreatus* appeared within 17-21 days after the inoculation. Further, previous studies depicted similar results to the current study that sugarcane bagasse and 100% corn cob required 44 days and 46.02 days respectively in order to obtain the first harvest of oyster mushrooms (Vetayasuporn, 2006; Hoa et al., 2015). In addition, Getahun (2011) reported that sawdust substrates needed 41 days for the primordia initiation due to their high C:N ratios (459.9). C: N ratio is the one of main reasons for the higher primordia formation and it is stated that the 20:1 C: N ratio of the substrate is crucial for a good number of primodia initiation (Chang and Miles, 1992). Moreover, Cueva et al. (2017) reported that lower nitrogen content in the substrates caused to variation in fruiting and spent a longer time to start the harvest. Thus, the substrates containing less C:N ratios (paddy husk and guinea leaves incorporated substrates - T2, T3, T4 and T5) spent less time (ranging between 7-11 days) for the primordia formation. Further, the substrates containing higher C: N ratios (Table 1) such as teak sawdust containing substrates required more time duration for the initiation of primordia and first harvest in contrast to the other substrates in the present study. Since the sawdust contains a high amount of lignin, low degradation of lignocellulosic substances of sawdust by *P. ostreatus* might be another factor affecting to the slower growth of mushrooms. According to the present study, substrates comprised only of sawdust (teak sawdust incorporated treatments and sole mango sawdust) have gained significantly longer duration (approximately 14 days) to initiate mushroom primordia. It would probably be due to poor water retention and quick drying than the other substrates under relatively high temperatures in the cropping room.

Interestingly, Das and Mukherjee (2007) reported that weed substrates significantly increase the protein content and reduce the production time of oyster mushroom due to adequate nutrients in the substrates as well as good physical properties favoring the fruiting body formation. The present study has also shown the same phenomenon that substrates consist with paddy husk (T3) and guinea grass (T5) produced primordia and fruiting bodies earlier than the other treatments. Moreover, Baysal et al. (2003) have reported that increment of the paddy husk amount in the substrates accelerate mycelium growth rate, pin head formation and fruiting body formation.

Mushroom cap diameter

The cap diameter of the oyster mushroom in eight different substrates is illustrated in figure 1C. The highest cap diameter was recorded from T7 (8.07 cm) which is statistically similar to all other treatments except T3 treatment (25% mango sawdust + 75% paddy husk) (7.47 cm). Patel and Trivedi (2014) have obtained similar results that range from 6.11cm to 7.43cm. Oyster mushrooms grown on T3 (25% mango sawdust + 75% paddy husk) substrate produced clusters containing more caps. However, their caps were in small size and poor quality compared to other treatments which may be due to the poor nutrient content and slower degradation of the constituents in the substrates. At the same time, sole mango sawdust, paddy husk and guinea leaves containing substrates produced clusters with larger and significantly higher numbers of caps than the teak sawdust-supplemented substrates. The present results agreed with Kimenju et al., (2009) findings, where cap diameter was very much dependent on the number of caps per cluster and the quality of the substrates. Thus, wider caps were produced in the clusters with few numbers of caps and good quality substrates produced large size caps compared with poor quality substrates.

Dry weight, fresh weight and biological efficiency

The current results depicted that different substrates significantly influenced the yield of *P. ostreatus* (Figure 1). The highest dry weight (46.18 g/kg), fresh weight (561.07 g/kg) and biological efficiency 56.11% were observed in T4 substrate (50% mango sawdust + 50% guinea grass). However, the fresh weight and biological efficiency obtained in the T5 substrate (25% Mango sawdust + 75% guinea leaves) were statistically similarly to the T4 substrate. Generally, the yields of *P. ostreatus* are varied due to the difference in bulk density and constituents of the substrates used (Dey et al., 2008; Samuel and Eugen, 2012). High cellulose content and lower C:N ratio may be the reasons for the highest yield in guinea leaves incorporated substrates. Overall, the teak sawdust-containing substrates showed the lowest dry weight, fresh weight and biological efficiency in contrast to the other treatments. Neupane et al. (2018) reported that slow mycelium running rate and low yield are possible due to presence of the polyphenolic compounds, low content of cellulose and poor water-holding capacity in the sawdust substrates. Further, Hoa et al. (2015) suggested that differences arisen in the yield and biological efficiency of *P. ostreatus* and P. cystidiosus were due to the variations of the physical and chemical composition of substrate formulas such as cellulose/lignin ratio and mineral contents, pH and EC of the substrate and especially C:N ratio. Moreover, it is reported that organic residues having a higher C:N ratio reduced the yield and quality of *P. ostreatus*, *P. florida*, and *P. sajor-caju* (Mintesnot et al., 2014; Hoa et al., 2015). Therefore, lower nitrogen content, higher lignin content, presence of phenolic compounds and more hard-wood portion are the factors that affected to the reduction of yield and biological efficiency of oyster mushrooms grown on the teak sawdust in the present study.

Further, average dry weight of the oyster mushrooms was higher than the values reported in the previous studies. For instance, the range of dry weight in different substrates was reported as 4.28 g - 29.98 g (Sarker et al., 2007) and 30.08 g to 35.15g (Biswas et al., 2016). Moreover, Miah et al. (2017) reported that the highest dry yield 18.88 g was obtained from the mahogany sawdust and the lowest from gamari sawdust. In 1998, Ahmed revealed that the lower diameter of caps led to the lowest yield and concluded that when the diameter of caps increased, the dry weight also increased. Thongklang and Luangharn, (2016) pointed out that the optimal substrate to cultivate *P. ostreatus* is sawdust + paddy husks with an average fresh weight of mushroom was 277.50 ± 79.74 g. However, comparatively higher amount of fresh weight of *P. ostreatus* mushroom was recorded during the current study, which accounted for an average of 480.93-503.73 g.

Previous studies have confirmed that the biological efficiency of oyster mushrooms depends on the different substrates and the values were reported as 39.55%-46.31% (Onyeka et al., 2018), 21.05%- 64.69% (Shah et al., 2004), 44%-103% (Vetayasuporn, 2006), and 31.56%-55% (Salami et al., 2017) which were aligned with the results of the present study that was accounted for 33.92% to 56.11%. Li et al. (2017) demonstrated that *P. ostreatus* gave higher yield and biological efficiency on mixed substrates. Similarly, most of the supplemented substrates in the current study showed a higher biological efficiency than the sole sawdust substrate, especially mango sawdust incorporated with guinea weed. Ashrafi et al. (2014) reported that spent mushroom substrates supplement with 60% sawdust + 20% wheat bran gained the highest biological yield (193 g/packet). Further, highest yield of *P. ostreatus* was obtained with the substrate composed of 20% paddy husk by Baysal et al. (2003). Das and Mukherjee, (2007) reported that supplementation of weed substrate with paddy straw increased the biological efficiency of mushrooms. Mintesnot et al. (2014) further found that *P. ostreatus* gave the highest biological efficiency when grown on *Parthenium hysterophorus* weed due to the high cellulose content and low lignin content of the substrates.

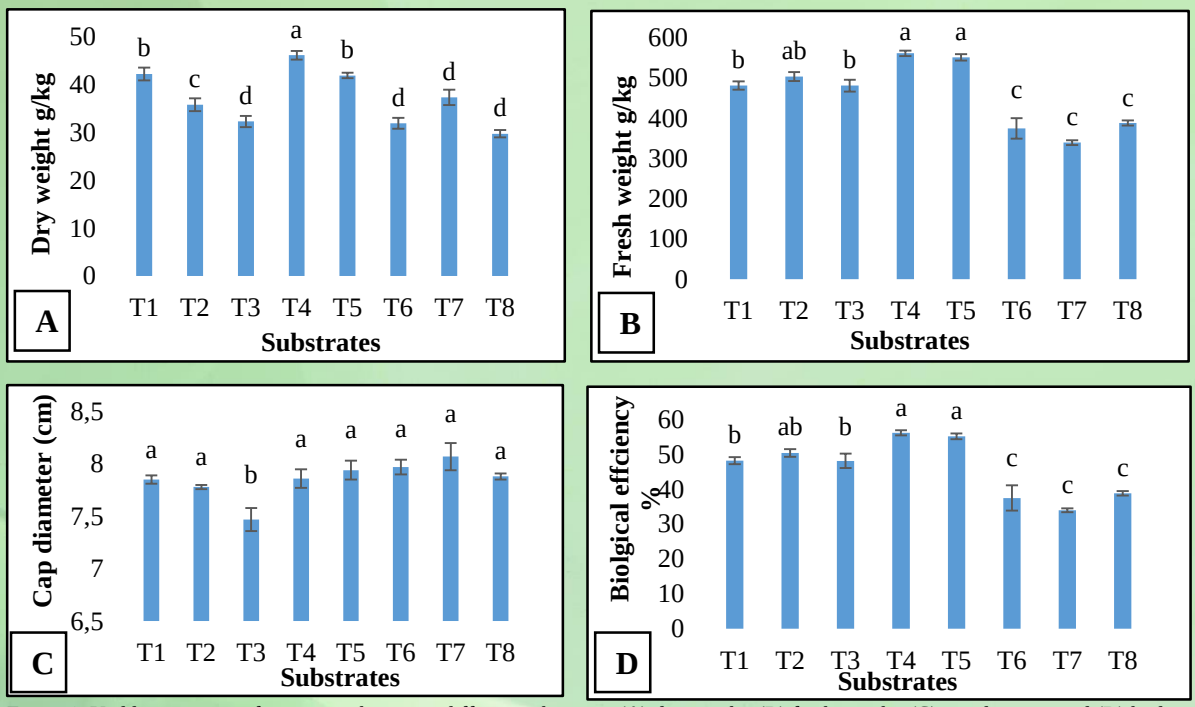

Figure 1. Yield parameters of oyster mushroom in different substrates: (A) dry weight, (B) fresh weight, (C) cap diameter and (D) biological efficiency (T1: 100% mango sawdust, T2: 50% mango sawdust + 50% paddy husk, T3: 25% mango sawdust + 75% paddy husk, T4: 50% mango sawdust + 50% guinea leaves, T5: 25% mango sawdust + 75% guinea leaves, T6: 100% teak sawdust, T7: 75% mango sawdust + 25% teak sawdust, T8: 50% mango sawdust + 50% teak sawdust; Means with different letters are statistically different at $p<0.05$)

Conclusions

Considering biological efficiency and other aspects, blending of guinea leaves or paddy husks in particular proportions was found to be more effective than the sole use of traditionally-used mango sawdust. Similarly, our attempt to incorporate teak sawdust did not yield better results. Therefore, integrated use of these low-cost and locally available materials will be a better option for the shortage of traditionally-used sawdust. However, in terms of feasibility, paddy husk is the most suited choice compared to guinea grass, since the preparation of guinea grass as a substrate necessitates the use of chopping machinery.

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