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Biomaterials from Spent Mushroom Substrate – a Sustainable Approach

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ABSTRACT

Spent mushroom substrate is not being effectively used and is true for Oyster mushroom spent substrate. The bio-based material that is produced after the growth of fungal mycelium in substrates such as paddy straw can be used in construction industry, for packaging purposes as a sustainable alternative to petroleum-based plastic packaging, as biodegradable containers and flower pots. Culturable mushrooms are good candidates for preparation of biomaterials from spent substrate. In the present study it was found the *Pleurotus ostreatus*, cultured from fruit bodies available in the market and *P. tuber-regium*, obtained from the wild, are suitable candidates for production of such bio-materials since they were found to colonize the substrate, paddy straw, and form a good composite material with the substrate. The identity of wild *P. tuber-regium* was confirmed by ITS rRNA gene sequencing.

Key words: *Pleurotus*, Oyster mushroom, Mushroom spent substrate, Biomaterials, Sustainable use

INTRODUCTION

Macrofungi (fungi with large visible fruiting structures) mainly belong to Basidiomycetes and some of them belong to Ascomycetes in Kingdom Fungi. Even though the number of macrofungi discovered so far is around 10% of the total fungi discovered, more are yet to be described. Although some of these are being used as potential candidates in food industry (Das *et al*., 2021) and Pharma industries (Gründemann *et al*., 2020), their use for the synthesis of biomaterials is just in the nascent stage.

Cerimi *et al*. (2019) reviewed the patents in the usage of fungi as source of bio-based materials and found that out of the 47 patents / patent applications claiming fungal materials for novel uses in the packaging, textile, leather and automotive industries, most of them were from United States, China and Australia, and 27 fungal species were utilized. Therefore, such studies in India will give encouraging results as many of the macrofungal cultures can be tested and brought into use with vast potential of untapped macrofungal resource. It creates a lot of opportunity for developing biomaterials from fungi in India since such work is meagre and the diversity of mushrooms is higher. Application as biomaterials include their usage in construction industry since fungi can aid in creating green construction industry, reducing pollution by reducing carbon emission as cement used in construction industry alone is responsible for 8% of global CO_2 emissions. Production of conventional construction materials consumes energy, and pollutes our air, land and water (Madurwar *et al*., 2013). Mycelium-derived materials have advantages including their low cost and low energy consumption in addition to their biodegradability and low environmental impact and carbon footprint (Jones *et al*., 2020). Fungal material can be used as organic insulation material with fire resistance that can be applied in home construction as a firewall panel. The mycelium-based composites grown on straw and hemp fibers serve as a natural insulator due to their low density and low thermal conductivity. In automobile industry, fabricated composite material based on fungal mycelium can be used to substitute petroleum-based products. Mushroom material can also be modified to have a wiring pattern for electrical circuit boards (Cerimi *et al*., 2019). Eco-friendly bio-composites derived from bio-based materials such as mushroom biomass could offer a sustainable alternative to petroleum-based plastic packaging in a wide range of applications. In cosmetic industry, *Ganoderma* sp. decreased the surface pigmentation by inhibiting the tyrosinase enzyme which is responsible for cutaneous pigmentation (Hsu *et al*., 2016).

To produce biomaterials, in this study we used spent mushroom substrate after harvesting the fruit bodies of *Pleurotus ostreatus* and *P. tuber-regium*. In many countries, the spent mushroom substrates (SMS) remaining after mushroom harvesting are often discarded as wastes (Chiu *et al*., 1998; Owaid *et al*., 2017) or used for cultivation of plants to increase the yield. But, in the present study we propose to use the mushroom spent substrate of *Pleurotus ostreatus* and *P*. *tuber-regium* for production of biomaterials.

MATERIALS AND METHODS

Sampling of Fruit-bodies

Fruit bodies of *Pleurotus ostreatus* (Jacq.) P. Kumm (white oyster mushroom) were obtained from the market (**Figure 1a**). Fruit bodies of *Pleurotus tuber-regium* (Fr.) Singer was sampled from Marakkanam, region adjacent to Puducherry (**Figure 1b**). Fruit bodies were brought to the laboratory in sterile polythene bags. During collection photographs of fresh specimens were taken, characters of fresh fruit bodies such as colour (Kornerup and Wanscher, 1978), size, etc. (Senthilarasu and Kumaresan, 2018) were recorded in the field. The fruit bodies were cultured in Potato Dextrose Agar medium (Kirk *et al*., 2008). Then, the fruit bodies were dried and stored in airtight polythene cover with naphthalene balls after labelling, to carry out the microscopic analysis for identification.

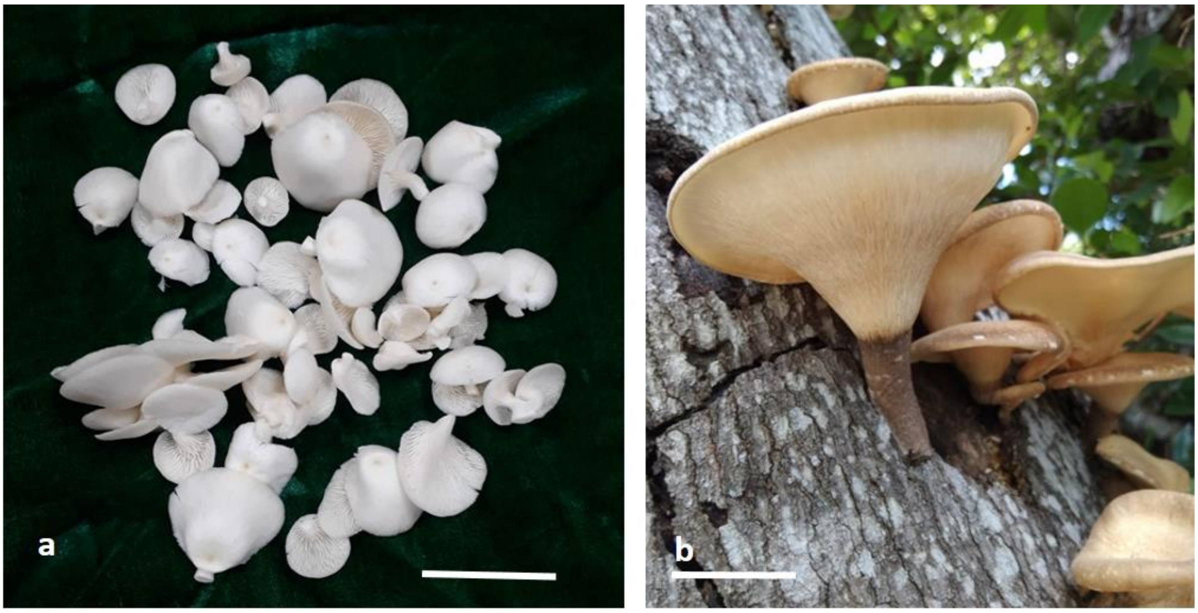

Figure 1: a, Fruit bodies of *Pleurotus ostreatus* obtained from the local market; b, Fruit bodies of *Pleurotus tuber-regium* growing on dead wood. Scale bar = 5 cm.

Fungal culture

The mycelial culture grown from the fresh fruit bodies of *Pleurotus* spp. were used to prepare the mother cultures in Sorghum grains. The mother culture was used for preparing bed spawn in Sorghum grains using the standard method (Gupta *et al*., 2016).

Substrate

The type of strain used for the synthesis of mycelium-based materials greatly influences the properties of the resultant composite. Mostly, the white-rot filamentous species are used to grow for the mycelium-based materials (Manan *et al*., 2021). In the present study, paddy straw was used as substrate. Paddy straw was chopped into 5-6 cm segments, immersed in water overnight and sterilized in an autoclave at 121^0C for 15 minutes followed by shade drying. *Pleurotus* spp. grain spawn was inoculated layer by layer in the paddy straw substrate contained in 16 x 12 inch polythene bags.

DNA extraction, PCR amplification and analysis

DNA extraction from fruit bodies of *Pleurotus tuber-regium* was done following Sawmya *et al.* (2013). The fungal 5.8S rDNA with its flanking ITS regions was amplified using the ITS primers. Primers ITS-1F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4R (5'-TCCTCCGCTTATTGATATGC-3') were used for amplification of fungal ribosomal RNA genes (White *et al*., 1990). PCR amplification was done following Kumaresan *et al.* (2021). The PCR products were purified and sequenced in ABI 3500 DNA Analyzer.

Phylogenetic analyses

Sequences with significant matches retrieved using NCBI Blast were selected and aligned using ClustalW (Thompson *et al*., 1994), checked visually and edited as required, and evolutionary trees were inferred using the maximum likelihood approach using MEGA 11 (Tamura and Nei, 1993; Tamura *et al*., 2021). Bootstrap analysis (500 replicates) was performed to calculate the branch support.

Fungal based biomaterial preparation

Once the fungal mycelium had fully colonized the paddy straw, and fruit bodies formed and harvested (in 4–5 weeks), the spent mushroom substrate (SMS) including the colonized paddy straw was compressed with or without mold based on the requirement. The compressed SMS was placed in hot air oven that was maintained at 60 ^{0}C. After drying at different temperatures, 60 ^{0}C was found to be suitable temperature for slow drying. Then organoleptic and physical properties of the fungal based material were carried out.

RESULTS

The mycelium of *Pleurotus ostreatus* and *P. tuber-regium* colonized the paddy straw fully in 15 days. In the case of *P. ostreatus,* buds started forming in the third week that further developed into mature fruit bodies (**Figure 2a**) and in the case of *P. tuber-regium,* buds formed during the 4^{th} week that further developed into mature fruit bodies (**Figure 2b**). After 4^{th} week of growth all the fruit bodies were harvested and the SMS was compressed. The SMS was observed under the stereomicroscope that showed hyphal threads around the paddy straw (**Figure 3a**). Initially the compressed substrate was tested for the suitable drying temperature when placed in hot air oven so that the substrate doesn't get brittle. The temperature when maintained at 60 ^{0}C was found to be suitable and the compressed substrate was free from moisture and neither brittle nor breakable (**Figure 4a, b**). The slow drying of colonized substrate led to fungal mycelium getting adpressed on the substrate (**Figure 3b, c**). Further, the spent substrate was compressed using two different molds, one structure similar to flower pot and other similar to small brick (**Figure 4b, d, e**). The spent substrate when dried for 2 days, 6 hours/day, become hard but not brittle. The flower pot structure (**Figure 4b, e**) weighed 340 g but was able to withstand weight of more than 25 kg. To ensure better shelf life, the composite material consisting of paddy straw and fungi were painted after drying. Further cow dung paint was also used (**Figure 4d, e**) making it as a product that would be easily biodegradable.

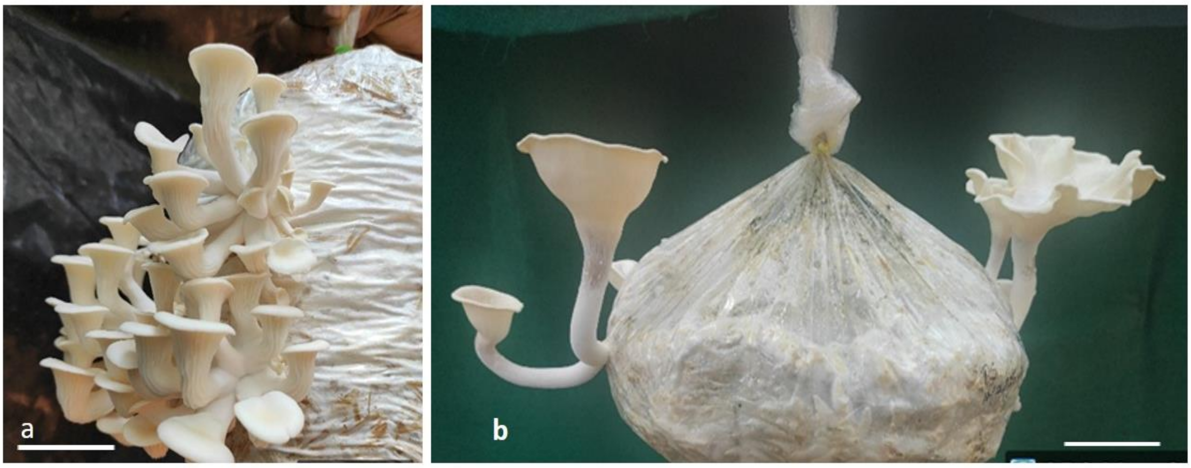

Figure 2: a**,** Fruit bodies of *Pleurotus ostreatus* grown in Paddy straw; b**,** Fruit bodies of *Pleurotus tuber-regium* grown in Paddy straw. Scale bar = 5 cm.

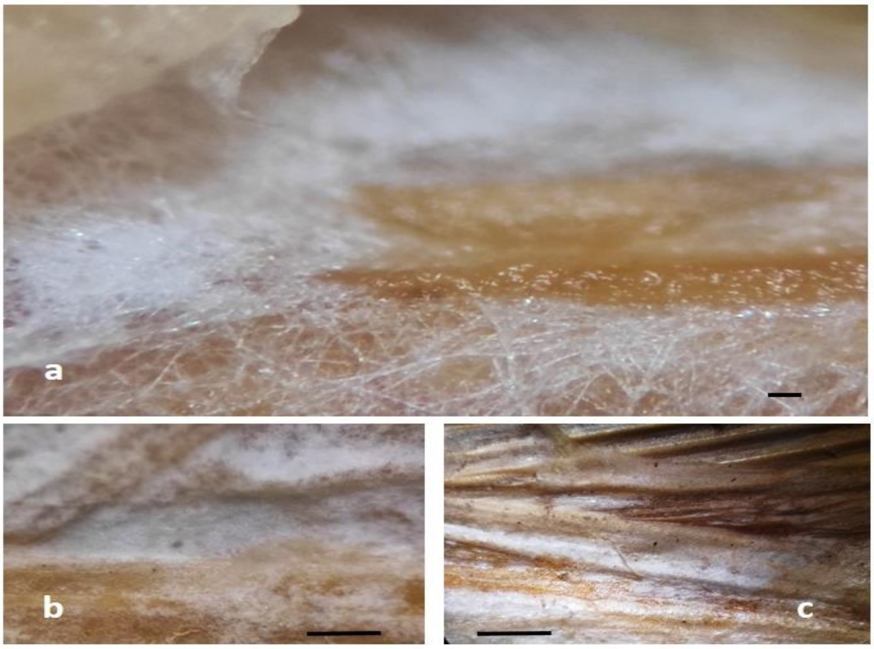

Figure 3: a, Hyphal threads around the paddy straw in SMS; b, c, Hyphal threads around the paddy straw after drying at 60^{0}C for 4 hours and 10 hours, respectively. Scale bar = 50 µm.

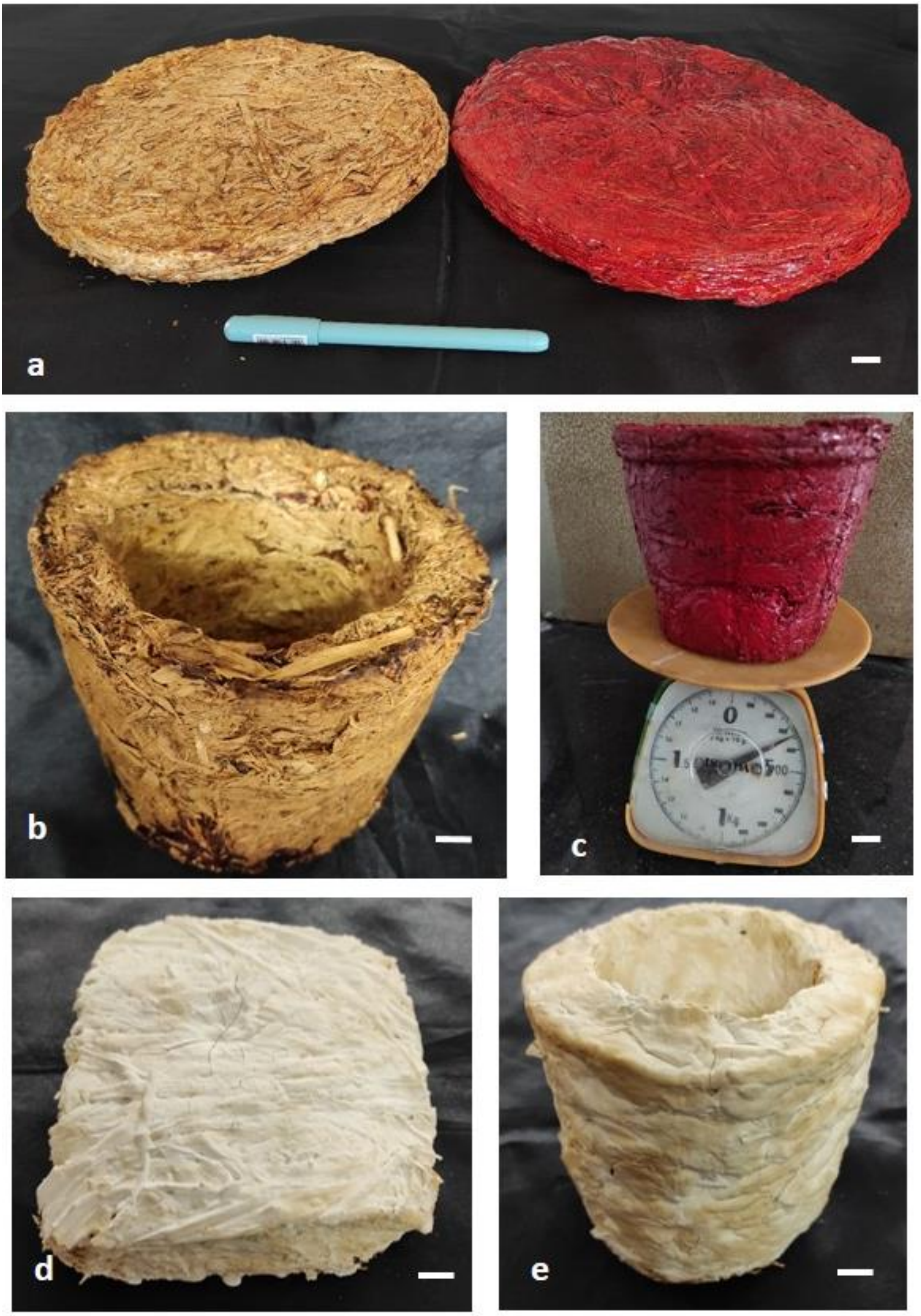

Figure 4: Products made out of biomaterial of spent mushroom substrate of *P. ostreatus* and *P. tuber-regium* fruit bodies grown in paddy straw. a, Product that can be used as packaging material; b, c, Flower pot made of SMS; d, Brick like structure; d, e, Products painted with cow dung paint. Scale bar = 1 cm.

To confirm the identity of *P. tuber-regium* collected from wild, Internal Transcribed Spacer rRNA gene sequencing was done. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model that showed similarity to other *P. tuber-regium* species (**Figure 5**). The tree with the highest log likelihood (-2502.65) is shown. The percentage of trees in which the associated taxa clustered together is shown below the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model (Tamura and Nei, 1993) and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 15 nucleotide sequences.

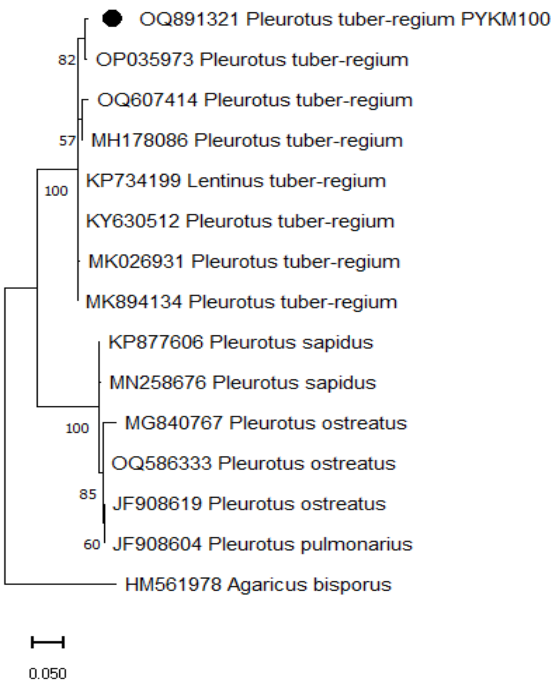

Figure 5: Phylogenetic relationship of *Pleurotus tuber-regium* (OQ891321) inferred from ITS sequences analysis by maximum likelihood method.

DISCUSSION

In the recent past, the use of living systems to material science for the synthesis of functional materials from biological resources is receiving considerable attention (Manan *et al*., 2021). For this, fungi seem to be an important and apt candidate not only because they have the ability for fast growth in synthetic and semisynthetic media but also the fungal mycelia have properties that include controlled and tunable during their growth, which would be ready to use without the need of expensive and sophisticated processing methods (Haneef *et al*., 2017; Alemu *et al*., 2022). This is especially true for macrofungi including gilled-mushrooms and polypores belonging to *Ganoderma, Polyporus, Lentinus, Pluerotus* etc. that can be grown in artificial media. The market for the packaging industry has increased in recent years as a result of globalization. The packaging materials currently being used include extruded polystyrene and expanded polyethylene foams that are primarily based on petroleum products. These materials are known to have disadvantages that include higher energy consumption during their production, difficulty in degradation process, and environmental pollution. Therefore, bio-based materials could provide an alternative to petroleum-based plastic packaging in numerous applications (Cerimi *et al*., 2019).

The amount of SMS produced is 3-5 times more than the mushroom produced and it varies depending on the mushrooms and their substrate used (Andrews *et al*., 2021). This accounts for tonnes of SMS not utilized in an effective manner. Thus, making biomaterials from SMS will be suitable option. Regarding the physical characteristics of the biomaterials prepared (**Table 1**), the biomaterial showed properties that can be used in construction, packaging and recreative materials.

Table 1: Organoleptic and physical properties of biomaterials

Organoleptic/Physical property	Biomaterial
Odour	Earthy odour, not observed after panting
Touch	Rough, smooth after applying paint
Heat tolerance	Tolerates more than 100^0C heat
Fire tolerance	Not easily flammable
Ability to withstand weight	Cylindrical structures can tolerate more than 25 kg

Both the edible mushrooms P. *ostreatus* and *P. tuber-regium* have thick hyphal systems that not only give characteristic texture but the mycelium colonize and bind the substrate in such a way that they form good composite material with paddy straw or even other substrates. Possibly, along with the fungal mycelium, it is opined that, other microbes including bacteria and actinomycetes may play role in binding of substrate and more studies are required in this aspect.

CONCLUSION

Usage of sustainable materials, especially fungal based biomaterials, would reduce environmental pollution. Fungal materials i.e., including the substrate are natural polymeric composites that are low cost and can be modified based on the substrate used. The fungi used in the present study are easily available and fast growing that can be used for biomaterial preparation, further, modifying the substrate to suit the kind of biomaterial to be produced will ensure enormous possibilities in this regard. Explorations of novel mushroom and bringing them into pure culture will expand the scope of such work.

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CONFLICT OF INTEREST

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