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Agro Composite Waste: A Novel and Economical Substrate for the Production of Edible Mushroom

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ABSTRACT

Mushrooms are edible macrofungi grown in many countries using various agricultural wastes. Mushrooms transform lignocellulosic waste and residues into valuable bioactive compound which are very important in the food and pharma industry. The purpose of this study was to grow *Calocybe indica* on 5 different substrates (Type A, Type B, Type C, Type D, and Type E) prepared from agro-waste such as rice bran, wheat bran, cow dung, rice straw, and leftover orange residue after essential oil extraction. Type-C substrate showed the highest biological efficiency, giving an optimal mushroom yield of 16.049% w/w and also exhibited the highest nutritional value as follows; the highest phenol content was observed in the Type-D category and the lowest in Type-C category, where mushroom showed 2.11 GAE/100g phenol content. The Type-B category mushrooms show the highest carbohydrates, energy, and ah contents compared to other types category mushrooms. The Type-E category mushroom shows highest protein and fibre content. The Type-D category mushroom contains highest Phenol and moisture content. The Type-C category mushroom contain normal and low nutritional values compared to another category mushroom. Therefore, black soil in a trace amount along with orange residue would enhance the biological efficiency and nutritional profile of *Calocybe indica.*

Keywords: Mushroom, Nutritional content, Spawn, Substrate, *Calocybe indica,* Biological efficiency.

INTRODUCTION

India is one of the world's largest agricultural countries, producing approximately 620 million tonnes of agro-waste each year (Singh *et al.,* 2014). Orange peel is a high-value agricultural waste (Ayala *et al.,* 2021). Approximately 34% of orange production is typically made into juice (Inacio, 2015) and its peel is discarded as waste by the household and juice industries. Orange waste is considered an environmental risk (Shweta *et al.,* 2022). Orange peel waste is rich in many nutrients, and excess nutrients are difficult to dispose of as they cause leaching and are left in the fields as compost (Udayasimha *et al.,* 2012). Therefore, there is a need to discover new methods that are inexpensive and less polluting. Mushroom cultivation on fruit waste could meet these requirements (Nicolcioiu *et al.,* 2016). Orange waste is a low-cost alternative for mushroom cultivation (Fabiola *et al.,* 2015).

Mushrooms are high-quality nutraceuticals and are known for their biological properties (Halpern *et al.,* 2002). Mushrooms are used in ayurvedic and folk medicine (Adhikari *et al.,* 1981; Jitendra *et al.,* 2000). *Calocybe indica* contributes more than 85% of mushroom production, according to the ICAR. It is the most popular variety in restaurants and homes after button and oyster mushrooms (Sharma, 2015). It has a wonderful flavor, robust, white sporocarps, meaty, umbrella-like structure and long shelf life (Kumar *et al.,* 2013). It grows between 25 - 35 °C (Alam *et al.,* 2010; Sharma *et al.,* 2008). This fungus grows naturally in the plains of Rajasthan and Tamil Nadu (Doshi *et al.,* 1989; Krishnamoorthy, 2015). Due to the high content of protein, essential amino acids, fibre, unsaturated fats, and vitamins (Pehrsson *et al.,* 2003; Pavel, 2009), it provides a significant dietary and nutraceutical benefit (Chelladurai *et al.,* 2021; Sharma *et al.,* 2017) which could be directly employed in human nutrition and health (Valverde *et al.,* 2015).

There is a lack of sufficient knowledge regarding its use for the cultivation of *Calocybe indica* (Sangeetha and Rajappan, 2020). In view of this we present the best possible use of orange peel waste for the better yield, high nutritional value, and fast growth of *Calocybe indica*. The use of fruit waste not only augments the waste disposal problem but also enhances the nutritional value of edible fungi and is cost effective.

MATERIALS AND METHODS

Preparation of substrate: In this study, different substrates such as paddy straw (used as a primary substrate), wheat bran, rice bran, black soil, cow dung, and orange peel residue (pectin) were used as secondary substrate (subsidiary) to culture the mushroom. The paddy straw was cut into 4-5 cm in length (Sangeetha and Rajappan, 2020) and soaked in water overnight (20 hours), after overnight soaking, the excess water was drained to retain 60 to 70% of moisture level. The above-mentioned substrates were filled in 5 different polythene bags and autoclaved for 2 hours and allowed for cooling overnight.

Table 1: Composition of different substrates

Substrate Category	Composition
Type A	Paddy straw+ Orange peel residue+ Rice bran
Type B	Paddy straw+ Orange peel residue+ Wheat bran
Type C	Paddy straw+ Orange peel residue+ black soil
Type D	Paddy straw+ Orange peel residue+ Cow dung
Type E	Paddy straw+ Orange peel residue+ Cow dung+black soil+Wheat bran+Rice bran

All five substrates were filled into 5 different autoclavable bags (2 kg capacity) and sterilized for about 2 hours at 121 ^{o}C and 15 lb pressure then allowed for cooling overnight.

Cultivation of mushroom: 10 gms of seed inoculum (Jowar grains) of novel strain was spread over the mouth of the bag and it was sealed with the ring and cotton plug in the sterile condition. The bags were placed in the dark, at a temperature range of about 20 - 28 ^{o}C and 85% humidity was maintained by spraying water around the bag.

Figure 1: Spawn run (Mycelium observation)

After the completion of spawn run, the bags were cut open from the top and were cased. Casing mixture was prepared by using black soil and cocopeat in the ratio of 3:1 respectively. The pH adjusted to 7.9 by 1N HCL and 1N NaOH. The soil mixture was autoclaved for 90 minutes.

The well grown and matured spawn aging about 35 days were used for the soil bed preparation. The study revealed that the casing layer thickness of 2.5 cm was ideal for better yield of mushrooms, as it provide sufficient ventilation (Subramanian and Shanmugasundaram, 2015). Mushroom spawn bags were kept in low ventilated room. water was sprayed every alternate day.

Harvesting of fruiting bodies: It took about 15 days for mycelium to reach to top of the casing layer, the bags were watered regularly as the moisture and humidity is important. Similarly, diffused light is also important for the initiation and growth of the fruit bodies. Light should be provided for maximum duration during entire cropping period. Mushrooms of 6 - 7 cm diameter were harvested by light twisting without disturbing the casing soil.

Figure 2: *Calocybe indica* harvest from different substrates. **A**, Fruiting body of Type-E front view; **B,** Fruiting body of Type-E dorsal view; **C,** Fruiting body of Type-D**; D**, Fruiting body of Type-C; **E**, Fruiting body of Type-B; **F**. Fruiting body of Type-D

Productivity and biological efficiency: Fruiting bodies from every substrate category were harvested, counted and weighed. The biological efficiency was determined at the end of the harvest period using the formula:

Bio-efficiency (%) = Yield of fresh mushroom (g) / Total weight of dry substrate used (g) x100,

Statistical analysis: The results obtained were analysed using one-way ANOVA by Past Software version 2.16c to test significance of growth characteristics and biological efficiency and nutritional profiling on five different substrates of *Calocybe indica* at $p<0.05$.

RESULTS

The *Calocybe indica* mushroom grown utilizing the primary substrate wheat bran (Type-B) has shown the nutritional value as Energy (318.78 kcal/100 g), Carbohydrates (51.98 g/100g), Total Fat (2.74 g/100g), Protein (21.55 g/100g), Dietary fibre (10.68 g/100g), Ash Content (8.99 g/100g), Phenol Content (2.19 gGAE/100g), Moisture Content (1.57 %). The mushroom cultivated on the black soil (Type-C) as the primary substrate has the nutritional profile as Energy (317.28 kcal/100 g), Carbohydrates (49.98 g/100g), Total Fat (2.64 g/100g), Protein (21.12 g/100g), Dietary fibre (10.38 g/100g), Ash Content (8.78 g/100g), Phenol Content (2.11 g GAE/100g), and Moisture Content (1.39%).

The fruiting body grew on cow dung (Type-D) as the primary substrate has nutritional profile i.e., Energy (315.87 kcal/100 g), Carbohydrates (48.53 g/100g), Total Fat (3.11g/100g), Protein (23.44 g/100g), Dietary fibre (11.19 g/100g), Ash Content (8.11 g/100g), Phenol Content (3.58 g GAE/100g), and Moisture Content (1.69 %). Similarly, the mushroom grown on a combination of all substrates (Rice bran + Wheat bran+ Black soil + Cow dung) (Type-E), has shown the nutritional profile values - Energy (317.78 kcal/100 g), Carbohydrates (49.98 g/100g), Total Fat (2.94 g/100g), Protein (24.55 g/100g), Dietary fibre (11.68 g/100g), Ash Content (7.99 g/100g), Phenol Content (2.48 g GAE/100g), and Moisture Content (1.33%).

The protein content in the mushrooms ranged from 21.12 to 24.55g. The highest crude protein 24.55g was obtained in Type-E Category while Type-C category gives the least value of 21.12 g protein content. The present analysis for energy showed a range from 318.78 kcal/100g to 315.87 kcal/100g. The highest energy 318.78 kcal/100g obtained in the Type-B Category while the Type-D category mushroom shows the least value of 315.87 kcal/100g energy content.

The ash content observed was between 8.99 g to 7.79 g which indicates that the mushroom contains some nutritionally important minerals. The highest Ash content of 8.99 g is obtained in the Type-B category while the Type-E category mushroom shows the least value of 7.79 g ash content.

The amount of fat in the mushroom ranged from 3.11 g to 2.64 g. The mushroom fat content was low as compared to the carbohydrate and protein. The highest fat content 3.11 g is obtained in the Type-D category while Type-C category mushroom showed the least value of fat content (2.64 g).

The carbohydrates in the mushrooms ranged from 51.98 g to 48.53 g. The highest carbohydrates were obtained in the Type-B category while Type-D category mushroom shows the least value of 48.53 g carbohydrates.

The Moisture content in different categories of mushrooms ranged from 1.69% to 1.33%. The highest Moisture content was observed in the Type-D category while Type-E category mushroom shows the least value of Moisture content.

The content of dietary fibre in different category mushrooms ranged from 11.68 g to 10.38 g. The highest dietary fibre observed in the Type-E category and lowest in Type-C category mushroom showed 10.38 g dietary fibre.

The Type-B category mushrooms showed highest carbohydrates, energy and ash contents compared to other category. The Type-E category mushroom shows highest protein and fibre content. The Type-D category mushroom contains the highest phenol and moisture content. The type-C category mushroom contains normal and low nutritional values compared to rest of the categories.

The phenol content in the mushroom analysis is done by the Folin phenol method. The phenol content in different category mushrooms ranged from 3.58 gGAE/100g to 2.11 gGAE/100g. The highest phenol content observed in the Type-D category and lowest in Type-C category mushroom showed 2.11 gGAE/100g phenol content.

Table 2: Growth characteristics and biological efficiency of different substrate

Substrate type	Average spawn run (days)	Number of fruiting bodies	Average total wet wt.(g)	Diameter of fruiting body(cm)	Average pileus diameter in (cm)	Average stalk length in (cm)	Average yield dry wt. (g)	Biological efficiency (%)
Type A	10	-	-	-	-	-	-	-
Type B	10	2	28.17	1	3.1	4.4	5.205	6.505
Type C	10	1	69.493	10	3.5	6.1	10.007	16.049
Type D	10	2	35.82	5.85	2.1	7.05	4.345	8.277
Type E	16	2	24.78	4.75	2.5	5.1	4.492	5.722

Note: FB, fruiting bodies; Wt, weight; g, grams; cm, centimetre; %, percentage

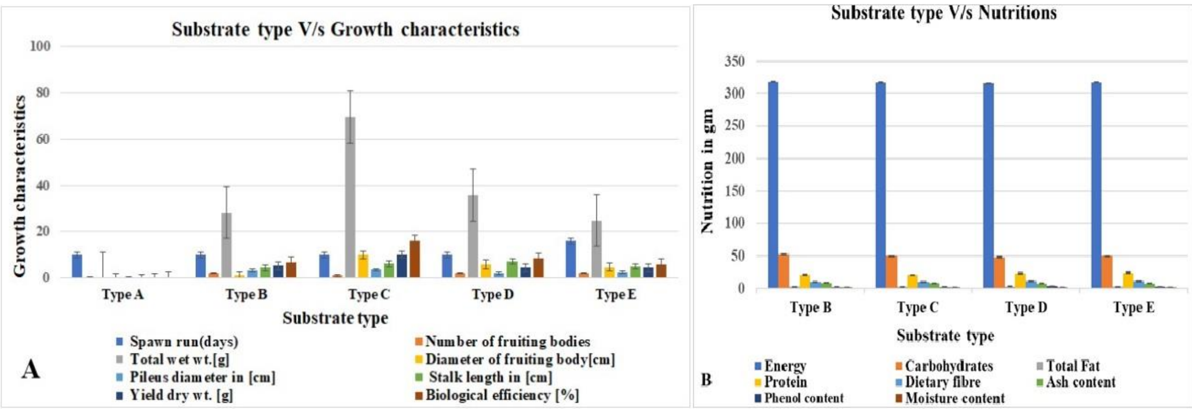

Figure 3: Comparison chart. A, growth characteristics and substrate type; B, nutrition and substrate type.

Table 3: The nutritional profiling of dried *Calocybe indica* grown in different substrates

S. No.	Parameters	Nutritional values in g and kcal				
		Type A	Type B	Type C	Type D	Type E
1	Energy (kcal/100g)	----	318.78	315.87	317.78	317.28
2	Carbohydrates (g/100g)	-------	51.98	48.53	49.98	49.98
3	Total fat (g/100g)	-------	2.74	3.11	2.94	2.64
4	Protein (g/100g) (N×6.25)	--------	21.55	23.44	24.55	21.12
5	Dietary fiber (g/100g)	--------	10.68	11.19	11.68	10.38
6	Ash-Content (g/100g)	---------	8.99	8.11	7.99	8.78
7	Phenol Content (g GAE/100g)	----------	2.19	3.58	2.48	2.11
8	Moisture Content in %	----------	1.57	1.69	1.33	1.39

Note: GAE, Gallic acid Equivalent; g, gram; %, percentage.

Table 4: Statistical analysis of Influence of growth characteristics and Biological efficiency and nutritional profiling on 5 types of substrates

Type of Characterization done for all 5 types of substrates (Type-A, Type-B, Type-C, Type-D, and Type-E)		Sum of Squares	Degree of freedom	Mean of squares	F-value	P-value
Growth Characteristics and Biological Efficiency Of 5 types of substrates	**Between sample**	341.54	3	113.847		
	Within sample	5292.69	28	189.025	0.6023	0.6189
	Total	5634.23	31			
Nutritional profiling of 5 types of substrates	**Between sample**	2.0318	3	0.677292		
	Within sample	330126	28	11790.2	5.745	1.0
	Total	330128	31			

Note: $p<0.05$ indicates the data significantly influenced the growth characteristics and nutritional profile of *Calocybe indica*

DISCUSSION

From the study conducted, in Type-A substrate (Rice bran) no pin head formation was observed hence no yield was obtained, this could be due to lack of nutrients in the substrate compared to other types. In Type-B substrate (Wheat bran) the pin head appearance was observed after 51 days of incubation and 2 fruting bodies were harvested after 10 days once the pinheads were appeared and this substrate yielded an average wet weight of 28.17 g and dry weight of 5.205 g and biological efficiency was recorded to be 6.505%. In Type-C substrate, (Black soil) the pin head appeared after 21st days of casing and harvested a single fruiting body after 10 days of pinhead appearance. The mushroom had average wet weight of 69.493 g and dry weight of 10.007g and biological efficiency was recorded to be 16.049%. In Type-D substrate, (Cow dung) the pin head appeared after 51 days of casing and total 2 fruiting bodies were harvested after 10 days of pinhead appearance. This mushroom had an average wet weight of 35.82 g and dry weight of 4.347 g and biological efficiency was recorded to be 8.272%.

The Type-E substrate (Combination of all categories), pin head appeared after 25 days of casing and total 2-fruiting bodies were harvested after 16 days of pinhead formation. This mushroom had an average wet weight of 24.78 g and dry weight of 4.492 g and the biological efficiency was recorded to be 5.722%. The maximum diameter of fruiting body and pileus was observed in Type-C substrate and the longest stalk length was observed in Type-D substrate. The growth parameters such as the length of fruiting body, weight of fruiting body was highest in Type-C substrate than Type-E, but Type-E substrate took short span of time to show the pin head appearance and fastest growth. The highest biological efficiency was recorded in Type-C (Black soil) substrate as it would have provided better nutrients to support the growth of the *C. indica*, hence, it is a better substrate for cultivation of *C. indica*.

Analysis of one-way ANOVA by Past Software version 2.16c to test significance of growth characteristics and biological efficiency and nutritional profiling on five different types of substrates of *Calocybe indica* at $p<0.05$ is represented in **Table 4**.

CONCLUSION

One of the greatest edible mushrooms that may be grown all year long in India's tropical climate is the milky mushroom. Its straightforward growth methods, minimal start-up costs and long shelf life are some of the qualities that make it a better option for both mushroom growers and customers. The success of milky mushroom cultivation is significantly influenced by the substrate utilized. Therefore, the goal of the current study was to determine the ideal substrate for maximizing yield and nutritional value. The findings of the study demonstrated that the optimal substrate was a combination of rice bran, wheat bran, black soil, cow dung, paddy straw, and orange residue. The maximum biological efficiency for optimum mushroom yield is 16.049% recorded from black soil substrate. Nutritious mushrooms can be made widely and inexpensively available to the general public to supply an adequate quantity of nutrition for a healthy and disease-free life.

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