

Original Research Article

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Characterization and Domestication of Wild Culinary Medicinal Mushroom *Pleurotus pulmonarius* from Assam, India

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ABSTRACT

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Considering the increasing demand of wild edible mushrooms and to sustain regular supply throughout the year, the indigenous mushroom species consumed by the ethnic tribes of Assam was collected from their natural habitat during the month of May, 2019. Germplasm of the species, identified as *Pleurotus pulmonarius* was isolated and successfully domesticated using sawdust and paddy straw as substrates. Complete substrate colonization at 2% spawn rate was observed after 2-3 weeks of inoculation. Biological efficiency of 32.8 ± 1.39 to 69.2 ± 0.72 was observed in domestication trials during the period of experimentation. Proximate analysis of the cultivated fruiting bodies revealed the presence of crude protein content 9.48g, crude fat 0.80g, carbohydrate 77.02g and crude fiber 34.30g/100g. No major difference in nutrient contents has been observed between cultivated and wild one. A year round cultivation trial was carried out to ensure regular source of nutrition and avenue for income generation. The successful domestication of this native species is a stepping-stone towards the cultivation of more wild edible species, consumed by the ethnic tribes of this region.

Introduction

Wild edible mushrooms have been consumed for thousands of years for its flavor, delicacy and medicinal importance (Rai *et al.*, 2005). During recent times they are considered as functional food due its incredible impact on human health and pharmacological activity (Kumar, 2015). Over the globe, more than two thousands species of mushrooms are found in nature. However, around twenty five species are accepted as food and some of these are cultivated commercially (Hussein *et al.*, 2015).

Oyster mushrooms are abundant in temperate and subtropical forested regions of the world. Indian Oyster, *Pleurotus pulmonarius* is commonly known as Indian, Phoenix, Italian or Lung Oyster. The medicinal attributes of this mushroom species has been investigated by numerous researchers throughout the world. Experiments suggest this edible mushroom species may be used as a therapeutic agent against a number of ailments (Smiderie *et al.*, 2008; Joseet al, 2002; Ibadallah *et al.*, 2015; Healing-mushrooms.net). Several wild edible mushrooms were domesticated successfully

over the last few years. Mushroom production technology is considered as one of the second most significant microbiological technology after the yeast (Xia *et al.*, 2016).

Assam, is a part of Indo Burma bio-diversity hot spot, located between 24°44' N to 27°45'N Latitude and 89°41'E to 96°02'E Longitude in the North Eastern front of India. The edaphic conditions and subtropical climate with annual average rainfall 1500mm and temperatures ranging from 6° - 38°C makes it a cradle of diverse fungal species. Wild edible mushrooms are highly coveted items of food for the ethnic tribes of this region. Rapid urbanization has resulted in the shrinkage of forest areas and erosion of forest wealth.

The rhythm of availability of wild edible mushrooms in their natural habitats depends on weather conditions. During rainy season, these edible mushroom species grows abundantly, however it is available in the natural habitat for a limited time span. None availability of these species in the dry season makes it an erratic source of nutrition. The ethnic tribes and village folk inhabiting the forest fringes have always harvested wild mushrooms from the forest for consumption and selling in local markets. In spite of having immense potentiality, domestication is not practiced due to unawareness of cultivation technology.

In this study the authors have attempted to domesticate traditionally preferred native oyster mushroom species to make it available as a regular source of nutrition and revenue. With this objective an experiment was designed for round the year cultivation of this wild edible strain in ambient conditions using locally available material as substrates. This study includes details of the collected species, cultivation methodology and biochemical composition of the species.

Materials and Methods

Collection and Isolation

The edible mushroom was collected during the month of May, 2019 from deforested area of Biswanath district of Assam. The mushroom was found growing in clusters on wood shavings under a felled tree. The collected sample was put in a paper bag and labelled with location, date and other information for laboratory work. Near by villages were visited to collect information about the desirability among the people. The mushroom was photographed in the natural habitat during the survey and some of the collected fruiting bodies were preserved in kew cocktail solution for future reference (Courtenay, 1982). Pure culture was isolated from the collected fruiting body by tissue culture method using Streptomycin (0.01%) amended potato dextrose agar (PDA) medium. Mother culture was obtained by subculturing mycelial fragments onto PDA slants and maintained at 4°C for further study. Various macro and micromorphological study were carried out in the laboratory. Cultural characteristics such as color, texture, growth rate, of the isolate grown on PDA were studied. The fungus was confirmed as *Pleurotus pulmonarius* (Kuo, 2017; Junior, 2010).

Morphological Characterization

Detailed macro morphological features such as colour, shape, size of the pileus were observed in fresh sample. A small portion of internal tissue of the fruiting body was mounted on a glass slide, in lactophenol cotton blue to observe microscopic features. Micro morphological characteristics were observed under a Zeiss AX10 microscope. All measurements (macroscopic and microscopic) were taken with the help of Image J software. Cultural characteristics such as colour,

texture, density, aerial hyphae, zonation and margin of the fungal colony grown on PDA medium were observed. Growth rate of the of the isolate grown on PDA medium was calculated according to the formula $GR = \frac{r_n - r_2}{t_n - t_2}$ where GR is growth rate (mm/day), r_n and r_2 is diameter (mm) of the colony on 7th and 2nd day of incubation and t_n & t_2 represents the growth period 7th and 2nd days (Guadarrama-Mendoza et al, 2014).

Proximate composition and Elemental analysis

Analysis of the proximate chemical compositions (Moisture, ash, crude fiber, crude protein and crude fat) and analysis of minerals compositions, which include Magnesium (Mg), Manganese (Mn), Calcium (Ca), Nickel (Ni), Copper (Cu), Potassium (K), and Iron (Fe) were conducted at NABL Laboratory, Tezpur University, Tezpur, Assam.

Determination of Energy value

Energy value was calculated using the formula: Energy value (kcal/100g) = (crude protein x 4) + (Total carbohydrate x 4) + (Crude fat x 9) (AOAC, 1990).

Cultivation and data collection

Cultivation was done in every month from June 2019 to May 2020 at ambient conditions.

Spawn preparation

Mushroom spawn was prepared according to Penn State Spawn Lab Procedures with slight modifications. In brief, parboiled wheat grains were mixed with CaCO₃ (2%) and CaSO₄ (0.5%), bottled and sterilized at 121°C for 15 minutes. After sterilization it was inoculated aseptically with fresh culture and incubated for mycelial colonization at 28±2°C.

Cultivation method

Mixture of agricultural waste (paddy straw) and sawdust along with additives such as wheat bran (2%) in the ratio 4:1:0.5, CaCO₃ (2%) and CaSO₄ (0.5%) were used as substrate. Moisture content of the substrates was adjusted to 60% approximately. One kg of prepared substrate was put in polypropylene bags and autoclaved at 15lbs PSI for 1 ½ hours (Adewoyin, 2018). After cooling, the sterilized substrate bags were spawned @ 2% and transferred to a dark room for colonization. After completion of spawn run, holes were cut at the sides of the bags and transferred to a well ventilated cropping room.

Data collection

Total yield, days taken for completion of spawn run, appearance of pinheads, date of harvest were recorded. The maximum and minimum temperature and humidity of the incubation and cropping room was recorded daily with the help of Alliance Digital Thermo Hygrometer. Light intensity in cropping room during pinhead formation and maturation phase was measured with lux meter LX-101A. Biological Efficiency (BE) was calculated according to the Chang *et al.*, 2004 by the formula:

$$BE = \frac{\text{Weight of fresh mushroom (g)}}{\text{Weight of dry substrate (g)}} \times 100$$

Statistical analysis

Standard Deviation of cultivation data and preparation of vertical column bar graph was done with GraphPad Prism5 software.

Results and Discussion

The species *P. pulmonarius* is a summer variety, found growing during the month of

May. Pileus measured 4–15 cm wide, fleshy, lung shaped (Fig 1), white with yellowish brown center, surface is smooth, convex and moist when young, on maturity pileus flattened, upward curved, became rugged and dry. Margin of the pileus was smooth in the young stage, latter becoming wavy and lobate. Flesh was white and colour did not change when cut. Gills off white, crowded, decurrent, with smooth edge becoming yellowish when dry (Fig 2). Stipe 0.5-2 x 0.7-1.5 cm long, eccentric, rudimentary, round, solid, white and smooth. Basidiospores 5.01-9.04 x 2.39-3.54 μm , cylindric to ellipsoid, smooth (Fig 3). Spore print white.

Colony morphology

The colony of the native strain, on PDA medium, was floccose, no zones, white, with abundant aerial hyphae and had irregular margin. No exudates were observed (Fig 4). Reverse of the colony was off white (Fig 5). The isolate grew at a steady rate of 8.75 mm/day up to the 5th day and at 16 mm/day from 5th to 8th day. The overall growth rate was 13.8 mm/day (Fig 6).

Proximate and Mineral analysis

The result of the Proximate analysis of domesticated and wild mushroom species are presented in the table 1. Domesticated mushroom showed appreciable amount of total carbohydrate (77.06g), fiber (34.30g), protein (9.48g), ash (1.28g) and very low fat (0.80g) contents (g/kg) on dry weight basis. The Calorie value of this domesticated mushroom was higher than the wild mushroom at 353.36 kcal/100g and 274.7 kcal/100g respectively. The wild specimen had higher protein (12.9g) and fat (1.5g) contents (g/100g), the carbohydrate content (52.4g) was lower than the cultivated strain. In the native strain significant amount of fiber has been observed which indicated that the

strain could be a good source of dietary fiber supplement. Mineral analysis revealed a high level of K (2474.76mg/100g) followed by Mg, Na and Ca. The lowest concentration among the major elements (K, Mg, Na & Ca) was Ca at 6.77%. Trace elements iron (Fe), manganese (Mn) and copper (Cu) were also detected at the concentration levels 3.89, 3.28 and 2.05 mg/100g, respectively (Table 1).

Cultivation

Oyster mushrooms can grow in a wide range of agricultural wastes and this strain is no exception. From an economic view point rice straw, being the most abundant agri-waste of this region, was chosen as substrate for cultivation. As the wild mushroom was found growing on wood shavings in their natural habitat, saw dust was incorporated at 25% along with paddy straw. Polypropylene substrate bags (30x25cm) were prepared in three replications during the 1st week of every month. The experiment was done in three replications.

Spawn run and appearance of fruiting body

The data of different attributing parameters during the period of cultivation were recorded and analysed (Table 2). The ambient temperature and relative humidity of the incubation room ranged from 19.7°-36.8°C and 33%-88% respectively. The cropping room ambient temperature and relative humidity during the period of experiment ranged from 14.6°-37.4°C and 33-88%, respectively.

Completion of spawn run or colonization of substrate bags, which ranged from 13-15 days, were not affected by temperature and humidity variations. Days taken for pin head or primordia formation differed with variations of temperature. During the summer

months (June-September) when the temperatures ranged between 34.1 to 37.4°C pinheads appeared in 7-9 days. During the winter months (November-January) when minimum temperatures ranged between 14.6 to 16.8°C pinheads took 23-34 days to appear. Pinheads were white and appeared in bunches (Fig. 7). The period of maturation i.e. from pinhead formation to harvesting depended on the relative humidity conditions. During the monsoon season when the humidity was high, the maturation period varied from 5-8 days (Fig. 8). However during the winter months when low humidity conditions prevailed, the

harvesting time was 3-4 days. The cropping cycle or the duration from inoculation to harvest of 3rd flush ranged from 57-102 days. It varied inversely with temperature, requiring less than 60 days from May to August and taking as long as 102 days in December, when the temperature decreased. From May to September when temperatures ranged from 31.5°- 37.4°C the biological efficiency (BE) ranged between 54.3±0 to 69.2±0. With the decrease of temperature during the winter months, October to January, the BE reduced to 32.8±1.39 to 34.6±1.44 (Table 2).

Table.1 Proximate and mineral composition of *P. pulmonarius* (dry wt basis)

Proximate analysis			Mineral content of domesticated sample	
Parameters	Domesticated (g/100g)	Wild (g/100g)	Elements	Quantity (mg/100g)
Moisture	11.37	15.2	Potassium	2474.76
Crude protein	9.48	12.9	Magnesium	193.25
Crude fat	0.80	1.5	Sodium	118.65
Ash	1.28	0.9	Calcium	6.77
Crude fiber	34.30	30.8	Iron	3.89
Total Carbohydrate	77.06	52.4	Manganese	3.28
Calorie value (kcal)	353.36	274.7	Copper	2.05

Table.2 Parameters of cultivation trial

Month	SR	PH	CC	BE	Temperature (°C)		Humidity (%)	
	(days)	(days)	(days)	(%)	Min	Max	Min	Max
June	15±0.0000	7.3±0.5774	59.3±2.3094	69.2±0.7217	26.1	34.1	57	88
July	15±0.0000	8.0±0.0000	58.7±1.1547	66.5±0.9014	27.3	35.8	51	85
August	14±0.5774	8.3±0.5774	57.7±1.1547	62.2±0.8780	26.9	37.4	52	85
September	15±0.0000	8.7±1.1547	62.7±2.8868	54.3±0.6292	26.1	35.2	52	85
October	14±0.0000	19.3±1.1547	87.0±2.6458	36.2±1.1273	19.7	33.7	45	80
November	14±0.0000	25.3±1.1547	95.0±3.0000	32.8±1.3919	15.3	27.4	45	80
December	14±0.0000	34.0±0.0000	102.7±1.5275	34.2±1.4434	14.6	26.1	44	78
January	15±0.0000	23.0±0.0000	82.3±0.5774	34.6±1.4434	16.8	22.5	33	77
February	14±0.0000	19.3±0.5774	69.7±2.0817	41.8±1.3769	17.8	25.7	36	73
March	14±0.0000	17.3±0.5774	65.7±1.1547	46.7±1.9094	20.1	29.1	39	76
April	14±0.0000	16.0±1.7321	62.0±3.0000	49.7±1.3769	23.4	30.7	41	79
May	14±0.0000	11.7±1.1547	57.7±1.5275	57.1±1.9094	26.3	31.5	71	88

SR = completion of spawn run, PH = appearance of pin head, CC= cropping cycle, BE = Biological efficiency

Fig.1 Mature sporocarp

Fig.2. Reverse side

Fig.3 Basidiospores

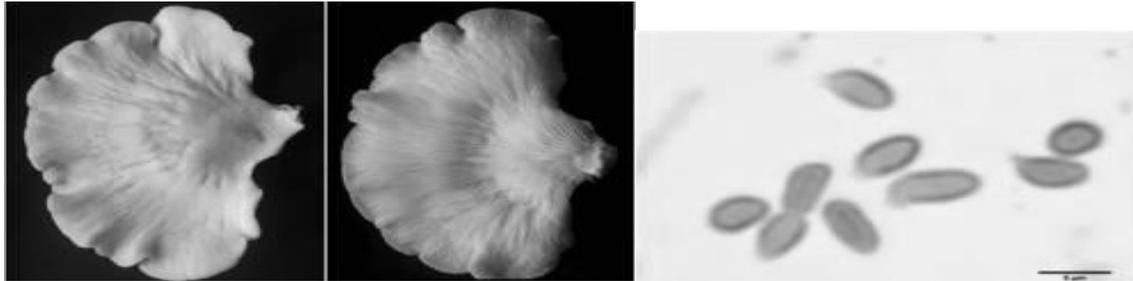


Fig.4 Fully colonized plate **Fig.5** Reverse side of colony

Fig.6 Growth kinetics

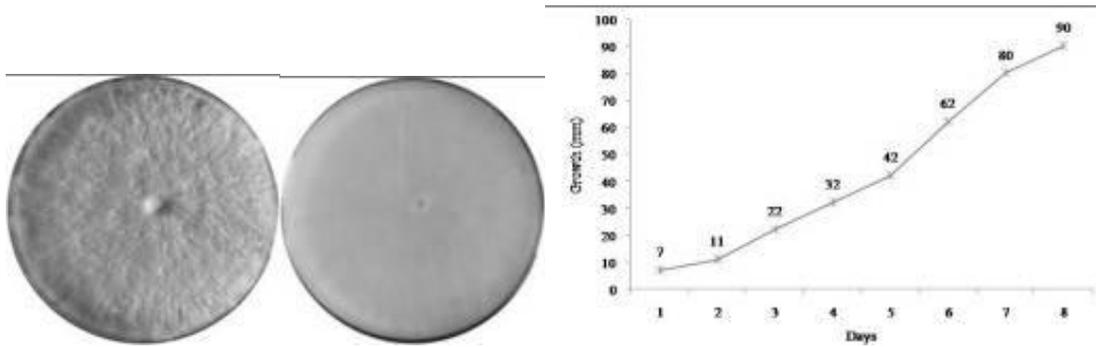
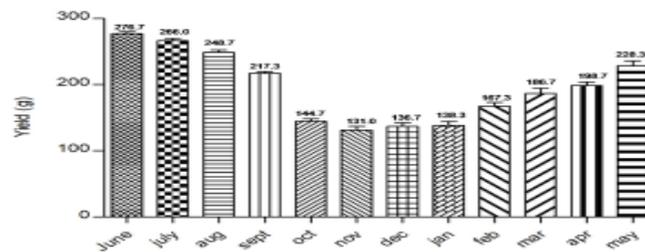


Fig.7 Appearance of pinheads

Fig.8 Mature fruiting bodies



Fig.9 Monthly yield data



Yield

The yield increased with the raised in temperature. The average yield per bag varied from 131.0-276.7g for the period of the trial with ambient temperature ranging from 14.6°C-37.4°C. The yield from May to September when the minimum temperature ranged from 26.1°C-27.3°C was between 217.3-276.7g. However during the months from October to January when minimum temperatures decreased ranging between 19.7°C -14.6°C reduction of yield (131.0-144.7g) was observed. The optimum condition for maximum yield of this strain was the months of June and July at 276.7g and 266.0g respectively. The temperature during this period ranged from 26.1°C-35.8°C (Fig 9). This strain showed a preference for warmer climate as reflected by the yield per kg of fresh substrate.

The pileus size 4-15 cm of this native strain is similar to the characteristics reported by Hemalatha *et al.*, (2018) where pileus size ranged from 5.2-16 cm, the stipe size however showed variations. The colony characteristics observed in the native strain was similar to the two reference strains investigated by Sobal *et al.*, (2017) with the exception that the native strain had a higher growth rate.

Results of the proximate composition are not consistent with the earlier work (Abulude *et al.*, 2018). Mushrooms are highly priced due to the presence of considerable amount of carbohydrate. The native strain seems to be richer in carbohydrate content than protein. Oloruntola and Omotosho (2019) observed 21.17% protein content on dry weight basis. Moreover this mushroom species is very rich in fiber. Which is important for diseases prevention and good bowel health. The high levels of major elements K, Mg, Na and minor elements Mn and Cu are in agreement with Abulude *et al.*, (2018) with the exception

of levels of Ca and protein content. The findings of biochemical analysis of this native strain would act as significant basis to determine its nutritional benefit. Considering the above results this mushroom is ideal as a functional food to promote human health. Although mushrooms are not fair substitute of animal protein, the native *P. pulmonarius* strain with 9.87% protein content could be suitable culinary to minimize the protein deficiency.

This native strain showed better tolerance to temperature and humidity variations at 14-37°C and 33-88% respectively as compared to the strain reported by Stamets (2000) at 18-29°C and 85-100% respectively. Bioefficiency of mushroom species is also dependent on strains. Myronycheva *et al.*, (2017) conducted experiments with six *P. pulmonarius* strains, one from IBK, Ukraine and 5 from Penn State University (USA). The findings of our study are consistent with the previous study with bioefficiency range 32.8±1.4% to 69.2±0.7% and vegetative growth range of 14 – 15 days (Myronycheva *et al.*, 2017). Cultivation of this strain is worthwhile due to its better price than other mushrooms as reported by Wu *et al.*, (2019). In the present investigation it has been observed that the native strain has excellent self-life, more than a week in the refrigerator without spoilage and can be dried easily. Moreover the native strain can be grown year round in wide ranging variations of temperature and humidity. The Same Chemical composition in cultivated and wild *P. pulmonarius* indicates that the cultivated *P. pulmonarius* is not less nutritious than the wild ones. This is similar to the earlier observation who reported presence of important nutrient in both wild and cultivated mushroom (Adedokun and Okomadu, 2016).

Fresh oyster mushrooms are sold @ Rs120-Rs150/kg in local markets during the months

of April–August. Dried oyster mushrooms are available throughout the year @ Rs 300/kg (Roy *et al.*, 2017). Many educated unemployed youth of remote villages in Assam were earning a decent living by mushroom cultivation. The produce could not be sold as markets were closed due to Covid-19 induced lockdown. The prevailing weather conditions hampered the drying of mushrooms, so the mushrooms rotted and caused considerable financial losses (Correspondent, 2020). Cultivation of this native strain could be an alternative option to mitigate such problems faced by the mushroom growers. Moreover mushrooms can also be preserved by making value added products i.e. mushroom chutney, candy etc and sold later.

In conclusion the present investigation reveals the new perspective of year round cultivation of the native wild edible strain. Since it is one of the expensive mushroom species, commercial production of this mushroom will help in upliftment of socio-economic status and sustenance of the rural populace. These important forest resources need to be explored extensively else it will remain hidden in the forest and become extinct.

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