Fazl Ali College Journal, ISSN: 2319-6769, Vol. 11 (2023) Pp.83-92

Domestication of Wild Edible Strain of *Pleurotus pulmonarius* (Fr.) Quél. (Oyster mushroom) from Mokokchung District in Nagaland State, India: A Prospect for Sustainable Livelihood.

Naomi* and T. Ajungla
Department of Botany, Nagaland University, Lumami 798627, Nagaland, India
*For Correspondence: naomipongen3@gmail.com

Abstract

Mushroom Cultivation is a sustainable farming activity that contributes to food security as well as the recycling of nutrients in the ecosystem. Mushrooms are saprophytic macrofungi that decompose dead biomass matters as substrates for its growth. Mushrooms have a high content of proteins, vitamins, and minerals. In Nagaland, exotic strains of Oyster mushrooms have been taken up for cultivation in the past few years. However, there has been no reports on cultivation of the wild edible mushrooms for economic purpose in the state, despite many species of wild edible mushrooms being reported as potential food source. In view of the huge number of wild edible mushrooms in the state, a study has been conducted with the objective of the domestication of wild edible mushrooms. In this study, a wild strain of Pleurotus pulmonarius Oyster mushroom was gathered from its natural habitat in Mokokchung, and the germplasm was isolated and spawn developed using standardised scientific techniques. Experiments were carried out under both controlled temperature and natural climatic conditions to assess growth performance and yield. The findings of this experiment demonstrated that domesticating the wild mushroom Pleurotus pulmonarius can help boost the region's economy and sustainable livelihood.

Keywords: domestication, germplasm, wild *Pleurotus pulmonarius*, substrates, biological efficiency, sustainable livelihood.

Introduction

Mushroom cultivation is emerging as an additional farming activity in many parts of the world. Mushrooms are basically saprophytic macrofungi that can grow on various dead and decaying biomass matters and thus, contribute to the recycling of nutrients in the ecosystem. Edible mushrooms are rich in proteins, minerals, vitamins, and fibre, low in calories and cholesterol. Mushrooms are considered nutraceuticals which may possess both nutritional and medicinal properties. There are about 2000 edible species of wild mushroom, only a few are widely accepted as food items and about a dozen of them have been domesticated and artificially cultivated globally; viz. Agaricus, Lentinula, Pleurotus, Auricularia, Volvariella, Flammulina, Tremella, and a few other species were brought under cultivation (Biswas et al. 2012).

Mushroom cultivation utilises various agricultural wastes and crop residues viz. paddy straw, wheat straw, sugarcane bagasse, cereal and

pulses husks and stalks, etc. as substrate media. The utilization of abundant agricultural wastes and residues is considered mushroom cultivation an eco-friendly farming activity. However, the method of mushroom cultivation is quite dissimilar compared with the other crop cultivation methods since mushrooms are non-photosynthetic and achlorophyllous, they grow on dead organic matter as decomposers in the absence of sunlight. Mushroom cultivation techniques involve the application of microbiological technology to develop the planting inoculum called the spawn. The study of the life cycle and ecology of the mushroom is an important aspect of the successful domestication of wild edible mushrooms under an artificial control environment.

It is recognised as landless—, indoor— and vertical farming, it provides ample opportunities like income generation for the landless farmers and women in particular. Mushrooms are grown at a very low cost since cultivation is done indoors with low requirements for water as compared to other crops (FAO, 2017).

As stated by Gilhotra. H., et al. (2023) mushroom cultivation not only generates employment but also helps in the socio-economic development of farmers and landless labourers. The commercial cultivation of mushrooms in the world started after World War II and in 2020, it achieved the figure of 42 million tonnes of mushroom production globally. In the present food economy, mushroom cultivation technique has been recognised as an important component of the expanding mushroom biotechnology industry.

Today, mushrooms are cultivated in more than 100 countries and China is the leading producer with a 95% share of the global market (FAOSTAT, 2022). The mushroom market in India is dominated by 5 genera viz. *Agaricus* (white Button mushroom), *Volvariella* (Paddy straw mushroom), *Calocybe indica* (Milky mushroom), *Lentinula edodes* (Shitake) and *Pleurotus* (Oyster mushroom). In India, due to significant variations in the climatic conditions and diverse geographical regions, the cultivation of different mushrooms is concentrated in different regions. White button mushroom is mainly grown in the northwest region, milky mushroom is prominent in the southern part, paddy straw mushroom is more localised in the coastal region of Odisha, and shitake and oyster mushroom are confined to North East India (Gilhotra H. et al. 2023). According to Royse et al. (2017), the oyster mushroom is one group of edible mushrooms commercially cultivated on paddy straw, wheat straw, sawdust, etc., and ranks second worldwide.

In the North Eastern region of India, oyster mushroom has the maximum share of mushroom production and consumption (Rajesha et al. 2018). In Nagaland, two exotic strains of oyster mushrooms belonging to *Pleurotus florida* and *Pleurotus sajor-caju* have been cultivated by the local farmers in small-scale farms as seasonal crops in both hills and plain areas. The ideal growing season in hills is from March to October and in plains from October to March (Rajesha et al. 2018). In Nagaland, particularly the tribal community considers mushrooms a rare delicacy and there is a regular collection of wild edible mushrooms during rainy seasons in the villages. Some of the species of wild edible mushrooms are available in the local market during the growing season at high prices. Ao and Deb (2019), reported 52 species of wild edible mushrooms including 3 species of wild oyster mushrooms belonging to *Pleurotus citrinopileatus*, *Pleurotus ostreatus*, and *Pleurotus pulmonarius* from Nagaland as

potential food sources. However, there has been no reports on cultivation of the wild edible mushrooms in the state for economic purpose, despite many species found in the forest. In view of the abundant wild edible mushrooms in the state, the present study has been conducted with the objective to see the feasibility of domestication of the *Pleurotus pulmonarius*, which is a common wild strain of oyster mushrooms in the region. *Pleurotus pulmonarius* is generally appreciated for its chemical constituents that possess many health benefits and have been considered as functional foods. Among the edible Oyster mushrooms, *Pleurotus pulmonarius* contain pharmacological properties such as antioxidants, anti-tumour, anti-cholinesterase, anti-inflammatory, reduced blood sugar level, and immune-modulatory composition (Zhang et al. 2016, Nguyen et al. 2016).

Study Area

The present study area is Mokokchung district, located in Nagaland. The GPS coordinates of Mokokchung district are 26.3220º N and 94.5153º E, annual rainfall ranges about 1600mm – 2500mm with a minimum temperature of 10º-15°C in winter and 25º-30°C in summer. The climatic condition of the region is suitable for the growth of mushrooms and thus serves as a reservoir of huge macrofungal forest bioresource.

Material

The mushroom material was collected from a wild habitat, grown naturally on the log of dead wood as shown in Fig 1. The GPS coordinates of the collection area are 26.317792º N and 94.521585º E. The mushroom fruiting bodies or the sporocarps were carefully detached from their substratum and kept in a box made of cardboard paper and brought to the laboratory. Taxonomic studies of the mushroom sample were done on the basis of morphological characteristics (fig.4 &5), whereas the edibility and safety of the mushroom sample for consumption were confirmed by local people who are involved in regular mushroom foraging. The in vitro culture was conducted in the Mushroom Spawn Lab in the Department of Botany, Fazl Ali College, Mokokchung in Nagaland.

The mushroom sample was identified as *Pleurotus pulmonarius* (Fr.) Quél, commonly known as the Indian oyster or lung oyster belongs to the family Pleurotaceae. The pileus is 3-10 cm across, convex, flat or depressed, the stipe is 1-4 cm long, the gills are close, the spore print is whitish, spore 7-11x2-3 µm, hyaline, centric, and ellipsoid.

Methodology

Isolation of germplasm and generation of spawn

The pure culture germplasm was isolated from a young fresh sporocarp by the tissue culture method following the protocol given by Hsu et al. (2018) with some modifications in the formulation of Potato Dextrose Agar culture (PDA) medium. The PDA culture medium was supplemented with antibiotics, adding 30mg of Streptomycin per 1000ml PDA medium. The addition of antibiotics helped in the prevention of bacterial contamination in the isolation of germplasm. In this method, the inner tissue of the sporocarp was excised and inoculated in the sterilised Potato Dextrose Agar (PDA) media in slant tubes to get the pure culture of the sample. The subculture was prepared by inoculating actively growing mycelia from the pure culture in PDA media in Petri plates (fig. 6) and incubated at 25± 2°C until a fully grown culture was obtained. Some of the pure culture in slant tubes was stored at minus 80°C in a deep freezer for future use.

Spawn Generation

Paddy grains were used as substrates for the generation of the spawns. The spawn generation was done according to the protocol described by Bora et al. (2020) with some modifications done in the process of making planting spawns. The planting spawns were directly produced from the mycelial subculture by skipping the Mother / Master spawn stage since it is required mainly for the production of large-scale spawns for commercial purposes. Following the method given by Bora et al. 2020, the paddy grains were washed and soaked in water for 6 hours and then boiled for 20 minutes. The excess water of the boiled grains was drained off in a mesh sieve and kept for about 6 hours to cool and evaporate up to 60 % moisture content. The boiled grains were supplemented with 0.5% calcium carbonate and 2 % calcium sulphate on a dry weight basis of grain to avoid clumping of grains during incubation. The supplemented paddy grains of 300 gm each were filled in polypropylene bags and sterilised in an autoclave machine at 121 ℃ for 90 minutes at 15 psi and allowed to cool at room temperature. The sterilised paddy grains were shifted to a laminar airflow chamber and inoculated with a bit of actively growing mycelia from the mycelial subculture in Petri plates. The inoculated bags were incubated at a temperature of 25± 2℃ in the BOD incubator until full colonisation of the mycelium. The bags were shaken at 5-6 days intervals to allow the mycelia to break and grow through the grains for uniform growth of mycelium in the spawn (fig.7).

Substrate preparation for cultivation

The domestication was done on paddy straw substrate. Paddy straw is available locally in the study area. The paddy straw is a suitable substrate for oyster mushroom cultivation. Pasteurization of the substrate was done by hot water treatment for 20 to 30 minutes. The paddy straws were packed in perforated sacks to allow water to drain. The moisture content of the substrate was tested by the palm press method and ensured 60-70 % moisture content. Inoculation and spawning were done as per the method given by Mshandete (2011), where the plastic bags containing 1 kg of substrates were inoculated by the layering method with 10 % spawn per dry weight of the substrate.

Experimental design for cultivation

The experiment was carried out using a group design method with two groups/sets (fig.3). The SET 1 group consisted of 6 replicates of inoculated substrate bags that were incubated in the incubation chamber at a controlled temperature of 25°C. The SET 2 group consisted of 6 replicates of inoculated substrate bags that were incubated in a dark room under natural environmental conditions without monitoring the temperature. The natural environmental temperature during the incubation period ranged between 20 to 27 ℃. The experiment was designed to study the influence of temperature during its mycelial growth period. The incubation chambers were disinfected with ethyl alcohol before keeping the bags and uniform darkness condition was maintained throughout the incubation period. During the incubation period, the mycelia growth initiated from the spawn grains and colonised the substrate materials, therefore this period is also known as the spawn running period. The initiation of the mycelia growth depends on various optimal factors like humidity, temperature, moisture content of the substrate, etc. The completion of the incubation period is indicated by the appearance of a white mycelial mat covering the entire straw in the bag. The bags that have developed tiny pinheads or primordia were shifted to the growing room. The relative

humidity between 70-90 % was maintained in the growing room, which was monitored by using a hygrometer. The light intensity in the growing room was adjusted by using a digital lux meter. The environmental condition required for growing *Pleurotus pulmonarius* is 21- 29°C temperature, relative humidity of 90-95%, and light of 500-1000 lux (Stamets, 1993).

Data collection and evaluation

The data collection was done on the growth performance and mushroom productivity. The overall evaluations were taken from the following parameters: spawn running period, induction of primordia, fresh weight of the mushroom yield, and biological efficiency. The Mean± Standard Deviation value was calculated from the data collected from the replicates.

The first phase of data collection was done on the spawn running period which was based on the number of days taken to complete the mycelial growth or mycelial colonisation. The second phase of data recording was done for the induction of primordia or the pinheads formation. The 3rd phase of data collection was the number of days taken for mushroom fruiting (Table 1).

Table 1. Evaluation of spawn running period, induction of primordia, and fruiting period

Replicates 6 nos.	Spawn running period Mean±S.D (days)	Induction of primordia Mean ±S.D (days)	Fruiting period Mean±S.D (days)		
			1st flush	2nd Flush	3rd Flush
SET 1	19.83±0.4	23.5±0.54	28.16±0.75	40.66±1.03	60.5±0.83
SET2	24.33±0.81	26.66±0.51	33.16±0.75	43.66±0.81	71.83±0.75

*SET 1 (incubated under control temperature at 25°C)

*SET 2 (incubated under natural environment)

The yield of mushrooms was recorded by taking the fresh weight of mushrooms harvested in the 3 successive flushes or fruiting periods and the harvesting period was continued till the 3rd flush (Table 2). The fresh weight of the mushroom was recorded from the first flush to the third flush during the experiment.

The time intervals between the two flush cycles were about 7-8 days. The harvested mushroom samples were dried in the hot air oven and recorded the dry weight. The dried samples were then stored at 4°C for analysis of their nutritional content. The fresh mushroom sporocarp normally contains about 90 % water. The biological efficiency (B.E.) percentage was evaluated as per the formula given by Chang et al. (1981).

$$B.E = \frac{\textit{Yield of the mushroom}}{\textit{Total weight of the substrate}} X\ 100$$

Table 2. Mushroom yield and Biological Efficiency (B.E)

Replicates 6 nos.	Mushroom yield (kg)			Total weight of mushroom (kg)	Biological Efficiency (B.E)
	1st Flush	2nd Flush	3rd Flush		
SET 1	2.31	1.35	0.6	4.26	71 %
SET 2	2.10	1.15	0.55	3.80	63.41%

*SET 1 (incubated under control temperature at 25°C)

*SET 2 (incubated under natural environment)

* Total substrate weight in 6 replicates SET is 6 kg

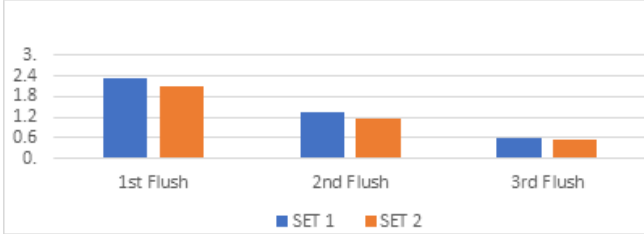
The evaluation of the percentage of biological efficiency (B.E.) of the mushroom cultivated under different environmental conditions and different substrates is important for mushroom economics.

Result and Discussion

From the data evaluation, it has been observed that the overall growth period of cultivation was completed in 60 to 70 days. Table 1 shows the average values for the spawn running periods obtained from SET 1 and SET 2 replicates, which were 19.83 and 24.33 days, respectively. The SET 1 replicates stored at an even temperature took less time to spawn than the SET 2 replicates kept in a natural environment. During the incubation phase, the average environmental temperature ranged from 20 to 27 °C. The cultivation took place between the months of August and October. The spawning phase for oyster mushrooms typically lasts 14 to 28 days, depending on the substrate and temperature (Chinda & Chinda, 2007).

After the completion of the spawn running phase, the mycelia intertwined into the mycelial cord to develop primordia within 3-5 days. The primordia are a group of mushroom young buds, commonly called pinheads, a number of primordia were developed near the area of the perforated holes on the surface of substrate bags. About 4 - 6 groups of primordia developed in each bag of replicates. Meanwhile, the induction of primordia is another major insight to determine the productivity of mushroom cultivation, the result is shown in Table 1. As soon as the primordia developed, the bags were shifted to the growing room and opened the bags to facilitate the free flow of air and also to allow the primordia to sprout into mushrooms. During the fruiting period, the growing room was maintained at 80-90% relative humidity and monitored regularly with the help of hygrometer readings. The moisture content in the substrate bag was also maintained at 60 to 70% water content. Regular watering was done during the fruiting period (fig.2).

Chart 1. Yield of mushroom in 3 successive flushes (weight in Kg)



The yield of the mushroom is quantified and is represented in Chart 1, showing the total weight (kg) of mushrooms obtained in 3 successive flushes of SET 1 and SET 2 replicates. The results have shown that both the replicates produced more or less equal amounts of mushrooms. However, the amount of yield decreased in the last flush.

This gradual decrease in mushroom production was due to a decrease in the amount of nutrients in the substrates. The substrate bag content also decreases in its weight. The total fresh weight of the fruiting body was converted into biological efficiency (B.E.) by calculating the ratio of the weight of the fresh mushroom to the dry weight of the substrate. The biological efficiency (B.E.) in SET 1 and SET 2 is 71% and 63.41 % respectively (Table 2). The biological efficiency of SET 1 is 71% which means a total of 710 gm of fresh mushrooms harvested in 1000gm of dry weight substrate in SET 1. Similarly, in SET 2 the B.E is 63.41 % which means 634.10gm of fresh mushrooms can be harvested in 1000 gm of dry weight substrate. The result obtained in both experiments is a positive indication for the domestication of this wild mushroom for economic uses.

Photo Section

Fig.1.Mushroom in wild

Fig.2.Domesticated mushroom

Fig.3. Incubated substrate bags

Fig.4. A sporocarp (dorsal view)

Fig.5. A sporocarp (ventral view)

Fig.6. Pure culture

Fig.7. Spawn bags

Fig.1 -7, Photographic representation of *Pleurotus pulmonarius* (Sources from present work)

Sustainable Livelihood Prospect

The economic aspect of cultivating wild oyster mushrooms for small-scale farming has been studied, which would be useful for revenue creation for local farmers. The assessment was based on the

current work's findings, as well as several references cited by Rajesha et al. 2018.

Table 3. Economics of Low-Cost Cultivation of oyster mushroom (* approx. cost price)

(A)	Non-recurring materials	Cost price	Remarks
	Construction of growing room (20X10 ft)	20,000	Capacity of 300 beds
	Chaff cutter	2,500	Manual operation
	Large cauldron/ pots	1,000	For substrate pasteurization
	Buckets and baskets	1,000	
	Water sprayer	200	Manual operation
	Sub Total (A)	**24,700**	
(B)	***Recurring materials***		**For one cropping (300 bags)**
	Paddy Straw (400 kgs)	1,000	From local Paddy field
	100 packets for 300 beds	3,000	300gm per packet
	Poly bags (30/40cm)-2kg	400	400 pcs
	Firewood	1,000	Locally available
	Sanitizers and disinfectant	300	
	Labor charges /Misc.	7,000	
	Sub Total (B)	**12,700**	
	Depreciation (Non-recurring expenditure) @ 10%	**2,470**	
	(C) Total Expenditure for one cropping (Rs.12,700+2,470)	**15,170**	
(D)	**Income**		
	Gross income from one cropping (B.E= 71%)	42,600	213 kgs from 300 bags @ Rs.200/kg
	Net income from one cropping	**27,430**	

Table 3 provides an economic estimate based on the current study and local context. The net income from single cropping with 71% biological efficiency is Rs. 27,430 (approx.), the amount that might be made in a single cropping cycle. Furthermore, because most raw materials, including paddy straw, firewood, and construction materials, are abundant in rural locations, farmers can further minimise their spending by obtaining the raw materials for free. In such a case, the net income or profit will grow.

Conclusion

Mushroom cultivation can be started and sustained with minimal infrastructure and financial expenditure. This study proved a straightforward method of cultivating a wild strain of oyster mushroom, as well as the economic component for income production. The current climatic conditions in the research area are also conducive to the growth of oyster mushrooms. Thus, based on the findings of this study, it is suggested that the domestically grown wild strain of oyster mushroom (*Pleurotus pulmonarius*) be considered a suitable crop for income production and adequate opportunity for sustainable life in the region.

References:

Ao T. and Deb C.R. (2019). Wild Mushrooms of Nagaland – An Important Bioresource. Studies in Fungi 4(1): 61-78.

Biswas S., Datta M., and Ngachan S.V. (2012). Mushrooms: A manual for Cultivation. Book Published by Asoke K. Ghosh, PHI. New Delhi.

Borah T.R., Singh A.R., Paul P., Talang H., Kumar B., and Hazarika S. (2020). Spawn Production and Mushroom Cultivation Technology. Book, ICAR Research Complex for NEH Region, Meghalaya, India, pp.46

Chang, S.T., Lav, O.W., and Cho. K.Y. (1981). The Cultivation and Nutritive Value of Pleurotus sajor-caju. European Journal Applied Microbiology & Biotechnology. 12, 58-62

Chinda M.M. and Chinda F. (2007). Mushroom cultivation for health and wealth. Apara Printers and Converters. Limited, Lagos. P.23-87.

Gilhotra H., Mamta and Paul D. (2023). Mushroom cultivation an opportunity for sustainable livelihood in the western Himalayan Region. Food and Scientific Reports ,4(3):1-7

Hasan M., Khatun M., Sajib M., Rahman M., Rahman M., Roy M., Miah M. and Ahmed K. (2015). Effect of Wheat Bran Supplement with Sugarcane Bagasse on Growth, yield and Proximate Composition of Pink Oyster Mushroom (Pleurotus djamor). American Journal of Food Science and Technology 3:150-157

Hsu C., Hammed K., Cotter V.T and Liao H. (2018). Isolation of Mother culture and preparation of spawn for Oyster Mushroom Cultivation. IFAS Extension University of Florida. SL 449

Kinge T. R., Adi E. M., Mih A. M., Ache N. A. and Nji T. M. (2016). Effect of substrate on the growth, nutritional and bioactive components of Pleurotus ostreatus and Pleurotus florida. African Journal of Biotechnology. 15(27), 1476-1486

Mshandete A.M. (2011). Cultivation of Pleurotus HK-37 and Pleurotus sapidus (oyster mushroom) on cattail weed (Typha domingesis)substrate in Tanzania. International Journal Research in Biological sciences . 1:135-144

Nguyen T.K., Im K.H., Choi J., Shin P.G. and Lee T.S. (2016). Evaluation of antioxidant , anti-cholinesterase and anti-inflammatory of culinary mushroom Pleurotus pulmonarius. Microbiology 44(4):291-301

Royse D.J., Rhodes T.W., Ohga S. and Sanchez J. E. (2004). Yield , mushroom size and time to production of Pleurotus cornucopiae (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates. Bioresource Technology. 91:85-91

Rajesha G., Bendagsenla , Singh M., Seyei A. and Rajkhowa D.J. (2022). Scenario of commercial mushroom production in Nagaland. Mushroom Research 31(1):113-117

Zhang S.,Liu B.,Liu J.H. and Wu Y.H. (2016). Cultivation technology improvement of Pleurotus pulmonarius. J. Anhui Agric. 44(36) 63-64