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Report on *in vitro* domestication and proximate analysis of wild strain of *Schizophyllum commune* (Fr.) from Mokokchung, Nagaland.

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Schizophyllum commune (Fr.) is acommon edible wild mushroom in Nagaland.The germplasm of the mushroom was isolated and the *in vitro* culture was developed on 3 different semi-synthetic culture media made up of the decoction extract from three locally grown tubers viz. sweet potato, tapioca, and potato with Dextrose and Agar. The culture condition was optimized at 25±2!, with the incubation period of 7 days for isolation and 14 days for sub-culture. The maximum radial growth of the mycelia was observed in the sweet potato Dextrose Agar medium. The spawns were produced on paddy grains as basal substrate. The domestication was tested on 3 different types of substrates, viz. paddy straw, cassava stem, and banana leaves. From which resulted the highest productivity in paddy straw with Biological Efficiency (B.E)of 18.14%. The result of nutrient analysis was found to be relatively similar in both the wild and domesticated mushroom samples.

Keywords: *In vitro* culture, local substrates, nutritional analysis, semi-synthetic media

INTRODUCTION

Nagaland is a state in the North East region of India, inhabited by 17 major indigenous Naga tribes. It lies within longitude of 25º6´E to 93º15´E and latitude 25º10´N to 27º4´N. In the present study area, the indigenous people belonging to the Ao- Naga tribe of Mokokchung district, utilizes the *Schizophyllum commune* (Fr.) mushroom as a special food item and it is called '*Kongyukonger'* in the local dialect.The climatic condition of the region is suitable for the growth of wild mushrooms, thus served as a reservoir of several wild edible mushrooms which were reported by Babhen *et al*. (2011), Kumar *et al*. (2013) and Ao and Deb (2018).

This wild mushroom is collected from the natural habitat and is often sold in the local market during the mushroom seasons, however, there has been no report on the domestication of this wild mushroom for economic purposes in the region.The *Schizohyllum commune*(Fr.)is a saprophytic lignicolous mushroom found to grow on dead and decaying wood of several phanerogams in natural habitat. It is widely distributed in many regions in the world.

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The common name of this mushroom is '*split-gill'* mushroom. The basidiocarp is a small and fan-shaped, white to grayish color, leathery texture with split gills. The basidiocarp is about 2-8 cm wide and 4-10 cm in length.

The *Schizophyllum commune*(Fr.) is popularly used as an edible mushroom by many ethnic people in Asian countries. In India, the indigenous tribes of North East region used this mushroom as delicacies.It was also reported that this mushroom has been used as nutraceutical for several diseases in oriental countries for many centuries (Hao *et al.* 2010).This mushroom has been reported to be a very good source of proteins, vitamins,lipids, and mineral elements (Adejoye *et al,* 2007). Kurnia *et al*., (2020), isolated the mycelia of this mushroom from rubber wood in Potato Dextrose Agar Media. Herawati *et al*., (2016), isolated this mushroom from the empty fruit bunches of palm oil and artificially cultivated using the bag log method. Ediriweera *et al*., (2015), reported the cultivation of *Schizophyllum commune (*Fr.) on paddy straws, banana leaves, and coconut leaves which were incubated at 28±2^{0}C. Debnath *et al*., (2020) cultivated on sawdust, rice bran, and a mixture of both and paddy straw. Bernabe-Gonzalez *et al*. (2022), reported the cultivation on

a substrate using corn stubble (*Zea mays* L), woody substrate (Dasanayaka and Wijeyaratne, 2017)and peanut shell (*Arachis hypogaea* L).

The objectives of the present study focused on three aspects viz. isolation of the mushroom germplasm in semi-synthetic culture media made up of locally grown tubers with Dextrose and Agar, domestication of mushroom on locally grown plants as substrate and evaluation of nutrient content of wild and cultivated mushroom.

MATERIALS AND METHODS

The sample was collected from a dead wood of an oak tree in Mokokchung, Nagaland (Fig.1 & 2). The GPS coordinates of the collection area are Latitude 26.220666" and Longitude 94.552317".The annual rainfall ranges from 1600mm to 2500mm with a minimum temperature of 10-15ºC in winter and 20-30º C in summer.

The *Schizophyllum commune*(Fr.)belongs to the Family Schizophyllaceae, and Order Agaricales of Basidiomycetes. Its sporocarp is about 2-7 cm wide, fan-shaped pileus, small hairs on the dorsal surface, white to grayish, stipe absent, gills present in the under surface, and the margins split through gills (Fig.3)

Pure culture of the mushroom

Culture of S. commune was obtaine bytissue culture methodand the pure culture was grown on 3 different types of semi-synthetic media made up of locally grown tubers viz. sweet potato, tapioca, and potato as the nutrient supplement. The media were adjusted to pH 6 by adding 0.1N Sodium Hydroxide(NaOH). The sterilization was done by autoclaving at 120 psi for 30 minutes. The autoclaved media were inoculated under sterile condition and incubated in a BOD machine at 25±2!for 7 to 14 days. The media formulations are given in Table. 1.

Spawn generation

The spawn was generated following the method given by Bora *et al*. (2020).Paddy grains were washed and boiled in clean water for 15-20 minutes. The boiled paddy grains were mixed with 2% $CaSO_4$ and 0.5% $CaCO_3$ on a dry weight basis. The mixture grains were taken 200 g in polypropylene bags and autoclaved at 22 psi pressure at 126 ^{0}C for 90 minutes. The sterilized paddy grain bags were inoculated with the actively growing mycelium from the culture plate and incubated at a temperature of 24 ± 2 ^{0}C to the produce planting spawns.

Substrate formulation for domestication

Three types of locally available agro-wastes,viz. Paddy straw, Cassava stems, and Banana leaves were selected for substrates in the experiment. The dried and senescence plants substrates were chopped and shredded into small pieces. The pasteurization of the non-composted substrates was done by thermal treatmentby immersing them in hot water.The experiment was performed on the Complete Random Design Method (CRD). In the experiment, each substrate was tested in 5 replications. Each replicate contains 200 g dry weight of processed substrate materials. Spawning was done at 20% of the inoculum per dry weight of the substrates. For the spawn running the temperature was maintained at 25^{0}C.

Proximate analysis

The proximate analysis of the mushroom was evaluated by the given protocols.

Moisture content

10g of fresh mushrooms of both wild and cultivated samples was weighed and dried in a hot air oven at 55 to 60ºC for 3 hours. Sample was then transferred to a desiccator to cool down. The process of heating and cooling was repeated till a constant weight was achieved. Moisture content was determined by the following formula following Raghuramulu *et al.*(2003).

$$\text{Moisture } \% = \frac{(W1-W2)}{W1} \times 100$$

Where , W1=initial weight of the sample and W2= Final weight of the sample

Ash content

The ash content of the samples was determined by AOAC protocol, (2000).

$$\text{Ash } \% = \frac{(W1 - W2)}{W1} \times 100$$

Crude Fibre

The crude fibre content was determined following the method of AOAC (2000).

Total protein

The protein content was measured by the method of Lowry *et al*.(1951).

Total lipids

The total fats were determined by the semi-continuous extraction by the Soxhlet method.

The weight of fats can be determined by subtracting the weight of thimble +glass wool + defatted sample

$$\text{Fat } \% = \frac{(\text{wt of fat})}{\text{wt. of sample}} \times 100$$

Carbohydrate content

Sample was hydrolysed in 80% Ethanol in boiling water bath for three hours. The hydrolysed sample was centrifuged at 8000 rpm for 3 minutes. The supernatant was collected and evaporated in a water bath at 80 ^{0}C.10 ml of D.W.was added and taken for analysis by the Anthrone method.

Determination of Biological Efficiency

The productivity was assessed by taking the fresh weight of the mushroom harvested in 3 successive flush cycles in all the replicates of each type of substrates (Fig. 5D-F).The biological efficiency of both substrates was evaluated as per the formula given by Chang *et al.*(1981).

$$\text{B.E.} = \frac{\textit{Yield of mushrooms}}{\textit{Total weight of the substrate}} \times 100$$

RESULTS AND DISCUSSION

In vitro culture

From the experiment, the data was collected on the basis of mycelial growth rate in *in vitro* culture. In this study, the culture was tested on 3 types of semi-synthetic culture media prepared from the tubers of *Ipomea batata* (sweet potato), *Manihot esculenta*(tapioca), and *Solanum tuberosum* (potato) with Dextrose and Agar in different ratios.

The data obtained in 3 types of semi-synthetic culture media is tabulated in Table 2**.** The growth of mycelia was highest in Sweet Potato Dextrose Agar medium (SPDA) media and relatively thicker and showed maximum radial growth as compared to the other two media within a period of 14 days incubation (Fig.4 A-C). The nutrient content in the SPDA media influences the mycelial growth to grow faster. The germplasm was subsequently sub-cultured for about 14 days and it was used for the generation of spawn.All the culture plates and slant tubes were incubated at 25±2!and the maximum growth of the mycelia was evaluated based on the mycelial density and the radial growth in the subculture plates.

The result obtained from this trail experiment is found to be very helpful for the local mushroom grower, to used locally grown tubers as a source of nutrient in the culture of mushroom mycelia.

Domestication

The domestication was tested on 3 types of substrates made up of locally grown agro-waste viz. paddy straw (*Oryza sativa*), cassava stem (*Manihot esculenta*), and banana leaves. The data was collected from the observation made on the following parameters, viz. spawn running period, induction of primordia, mushroom productivity, and biological efficiency (B.E.) (Table3)

The domestication was experimented on 3 types of agro-waste substrates,readings collected on 5 replicates, and the efficiency of the mycelial growth was evaluated by the observation of the rate of spawn running during the incubation period

Table 1: Semi-synthetic culture media ingredients formulation

Culture media	Ingredients (g/l)				
	Dextrose	Agar	Potato	Tapioca	Sweet potato
PDA	20	18	200		
TDA	20	15		200	
SPDA	15	18			200

* PDA (Potato Dextrose Agar), TDA (Tapioca Dextrose Agar), SPDA (Sweet potato Dextrose Agar)

Table 2: *In vitro* culture of *Schizophyllum commune* (Fr.) on three types of semi-synthetic media

Culture media	Germplasm isolation growth period (days)	Sub culture growth period (days)
SPDA	7	14
TDA	10	14
PDA	9	14

*SPDA (Sweet Potato Dextrose Agar Medium), TDA (Tapioca Dextrose Agar),PDA (Potato Dextrose Agar)

Table 3 : Effect of substrate on mycelial growth and primordia formation showing in days

Substrate	Spawn Running (days)	Primordia induction (days)	Flushing period (days)
Paddy Straw	*15.5±0.54*	2.5±1.14	24.8±0.83
Cassava stem	*17.2±0.70*	5.8 ±0.70	27±1.22
Banana leaves	*22.2±1.30*	4.8±0.83	35.8±1.3

Table 4 : Total yield and biological efficiency of mushrooms in 3 types of substrates

Substrate	Total yield in 3 flushes (g)	Mean±S.D	B.E %
Paddy straw	453.69	90.73±6.6	18.14
Cassava Stem	383.19	76.63±3.53	15.32
Banana leaves	184.7	36.94±5.3	7.38

in a dark room at a temperature of 26±2 ^{0}C. The results are given as mean value ± standard deviation (Table3). The spawn running period in paddy straw was*15.5±0.547 days, followed by cassava stem with 17.2±0.707 and slowest in Banana leaves with 22.2±1.3days.* The rate of mycelial colonization was found to be influenced by temperature and rate of substrate decomposition by the enzymatic action of the growing mycelia. In the substrate bags the mycelia forms a superficial layer made up of white cotton like appearance (Fig. 5 A). In the mycelial colonized substrate bags, more primordial formation towards the upper surface of the bag.Under cultivation, the primordia are seen to developed very prominently with a callus-like structure developed from thick mat of mycelia which eventually grow into mushrooms in a group (Fig.5.B), whereas, the popcorn-like primordia are developed from the thin or sparsely colonized mycelia, such primordia eventually developed into solitary or single mushroom sporocarp (Fig.5.C).

Fig.1: Study Area: Mokokchung district in Nagaland (source: www.veethi.com)

Fig. 2 : Wild *Schizophyllum commune* (Fr.)

Ediriweera *et al*. (2015) reported that the incubation of the substrate bag at 28±2 ^{0}C was successful. According to Rosnan *et al*. (2019), the optimum temperature for mycelial growth was 28°C. Those bags showing complete colonization of mycelia with the initiation of primordia are shifted to the Mushroom Growing Room. Relative Humidity of the growing room was maintained at a range of 70 -80 % and the substrate bed was also kept moist by watering regularly. The humidity

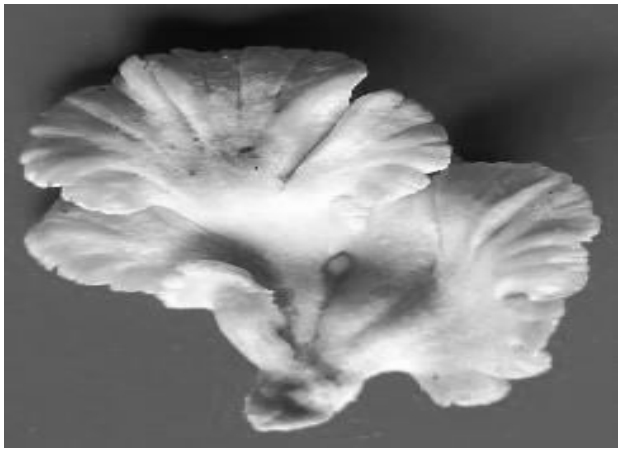

Fig.3 : Cultivated *Schizophyllum commune* (Fr.)

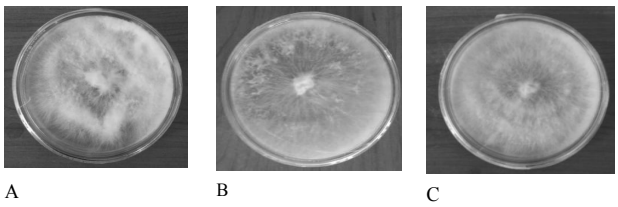

Fig. 4: Mycelia cultures in 3 types of Semi-synthetic culture media : SPDA culture medium (A), TDA culture medium (B) & PDA culture medium (C)

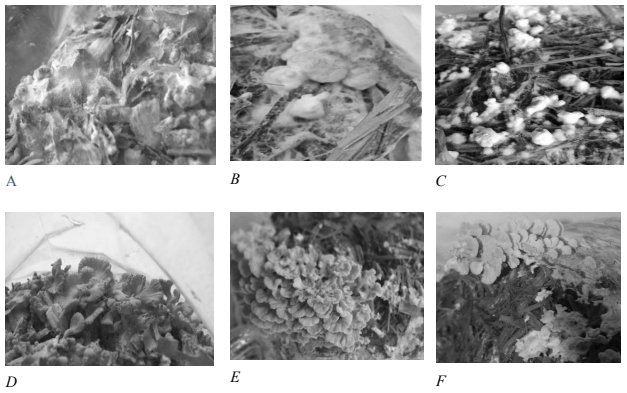

Fig.5: Mycelial colonization in Cassava stem (A), Callus like primordia in paddy straw (B), popcorn like primordia in banana leaves (C). Mushroom flushing period (D-F).

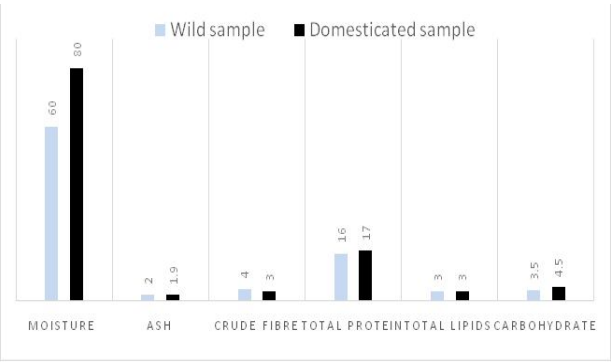

Fig.6:.Proximate analysis of wild and cultivated fruiting bodies of *Schizoplyllum commune* (Fr.)

is crucial in fruiting so it was regularly monitored by using a portable hygrometer to indicate the humidity inside the room.The bags were cut open near the primordia to permit the growth of the sporocarp without any obstruction. The induction of primordia is the sign of initiation of mushroom fruiting.The primordia induction and first flush were found to be faster in paddy straw, followed by cassava stem, and the last one in banana leaves. From this observation, the paddy straw substrate results in better performance.

Yield and Biological Efficiency

The total weight of all the mushrooms harvested from the 3 flushes cycle in the 3 types of substrates were recorded.Replicates of paddy straw give an average of 90.73±6.6g (fresh weight) mushrooms, cassava stem gives an average of 76±3.53g (fresh weight) and banana leaves give an average of 36.94±5.3g. The yield of the mushroom decreased from one flush to the other consecutive flushes, which is due to the exhaustion in the nutrient contents of the substrate.The growth period was continued for 3 times flushing in paddy straw and casava stem substrate bag, whereas in banana leaves substrate bags the mushroom ceases to produce fruiting after the first flush. In this investigation, the biological efficiency (B.E) of *Schizophyllum commune* (Fr.)cultivated on paddy straw is 18.14%, on cassava Stem is 15.32% and on Banana leaves substrate is 7.38 % respectively. The yield of the mushroom can be increased by adding supplements such wheat bran, rice husks etc. Table shows the yield of mushroom and B.E % corresponding to 3 harvests in 3 types of substrates. Figlas *et al.,* 2014, documented the yield of *Schizophyllum commune*(Fr.) on domestication as B.E % of 48.3 % on sunflower seed hull supplemented with 7.5% wheat bran. The Mushroom productivity was mainly dependent on the substrate type, spawning rate, and growth condition.The domesticated mushroom was found to be relatively more healthy, soft in texture and pinkish white in colour. Debnath *et al*. (2020) also reported the cultivation of *Schizophyllum commune* on agro-industrial waste.

Proximate analysis

The proximate contents of the *Schizophyllum commune* (Fr.) of wild sample and domesticated sample wereevaluated.The results are given in the (Fig.6).From the observation,it was found that the domesticated mushroom samples contain more amount of moisture than the wild mushroom sample, i.e. by 20 %,more , the reason may be because of the regular hydration of the mushroom bed under domestication. Whereas the wild mushroom has lesser moisture content as the sample was collected on a wooden log, which do not have the ability to retained water like the paddy straw in domestication. The amount of ash content of both the sample was similar amount of percentage, i.e. 2% in wild sample and 1.9 % in domesticated sample. Herawati*et al*. (2016) reported the presence of 2% ash content in *Schizophyllum commune* (Fr.).The crude fibre was more in wild sample than the domesticated i.e. 4% and 3% respectively. The dried mushroom materials were found to contain the total protein content of 16 % in the wild and 17.5 % in domesticated sample. The amount of protein content in this mushroom was found to be variable according to different workers. The same amount of total lipid contentwas present in both wild and domesticated mushroom.The ash content was found to be similar in both the sample. In mushrooms the total carbohydrates are present in the form of polysaccharides such as starches, glycogen and fibre. The present study shows that the total carbohydrate in wild is 3.5 % and the domesticated sample was 4.5%, however there are wide variation in the carbohydrate content reported by different workers.

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