

In vitro Cultivation of Newly Reported Wild Edible Mushroom *Volvariella bombycina* from Nepal

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Abstract

Wild edible mushrooms are becoming endangered all over the world. Very few wild edible mushrooms are found in natural habitat. *Volvariella bombycina* is an edible and medicinal mushroom. The mushroom was collected in natural habitat growing on *Populus* tree. Mycelium of the mushroom was developed in PDA slant tubes by tissue culture method, incubated at 25°C for 1-2 weeks. Spawn was developed in wheat grains after incubation at 25°C for 2-3 weeks. Substrates were formulated for the development of fruiting bodies by combination of paddy straw, saw dust and rice husk. Fruiting bodies of *V. bombycina* was cultivated in these substrates after incubation at 28 ± 2°C for 2-4 weeks. The work describes the optimized process for in vitro culture of wild edible mushroom *Volvariella bombycina*.

Key words: Mushroom; Wild; Edible; cultivation; *Volvariella bombycina*

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Introduction

Wild edible mushrooms possessing medicinal and nutritional properties have been collected and consumed by people since time immemorial [1]. Nepal is rich in biodiversity due to its complex variation in geomorphology and phytogeography [topology, climate and altitudinal]. It is known for being rich in mushroom diversity [2]. Due to this, the exploration for novel mushrooms is of paramount importance. Among such, one important edible and medicinal mushroom, *Volvariella bombycina* has just been recorded in 2016 by Adhikari M.K on *Populus* tree from Tribhuvan University, Kirtipur [6].

Volvariella bombycina [Schaeff.:Fr] Singer, commonly known as Silky agaric, Silky sheath, Silky rosegill, Silver-silk straw mushroom, Tree mushroom grows on *Populus* tree in Nepal and is distributed in China [3], North America [4], India [5], Nepal [6], Pakistan [7] and Korea [8]. *V. bombycina* is a tropical and subtropical species and belongs to the family Pluteaceae [6]. *V. bombycina* usually grows in a shady place on the rotting wood, leaf debris, rich agricultural soil especially in coffee and palm plantation [9]. It is an edible [9, 10] tasty, with a modest and pleasant flavor [11] with potential for commercial cultivation [13, 14]. The morphology of fruiting bodies of *V. bombycina*

are highly variable, suggesting a strategy for adaptation against environmental stresses caused by a range of extrinsic factors [3]. The life cycle, hymenial cell type, cystidia and basidia of *V. bombycina* are similar to that of *V. volvacea*. However, on contrary to *V. volvacea*, it requires slightly lower temperature for mycelial growth and fruiting starts from 26°C to 30°C [14]. *V. bombycina* produces various bioactive secondary metabolites; ergosta-4,6,8[14],22-tetraene-3-one, ergosterol peroxide, indole-3-carboxaldehyde, indazole [15], and isodeoxyhelicobasidin in liquid culture [16]. The mushroom possesses several biological properties such as antioxidative, antitumor, hypocholesterolemic [17] and antimicrobial properties [18].

Volvariella bombycina has a great commercial value all over the globe since it can be consumed as a substitute for meat because of its high protein content and for its enormous medicinal value [19]. Attempts for its' cultivation have not been reported from Nepal till date with few reported attempted from China [3] and India [19]. This difficulty in cultivation could be attributed to variability in nature of fruiting bodies [3]. Similarly, previous methods were not suitable for commercial cultivation. This research utilized the easily available local substrates such as paddy straw and others to

induce development of fruiting bodies from wild edible mushroom *V. bombycina*.

Materials and Methods

Collection, Identification and Handling of Fruiting bodies

V. bombycina was collected from the trunk of *Populus* tree found at Tribhuvan University, Kirtipur in paper tissue and cultured on the same day. The collected mushroom was identified by morphological and spore print characteristics. Some specimens were dried under natural light at room temperature for several days. Herbarium was prepared and deposited at National Herbarium & Plant Laboratories, Godawari, Nepal under Department of Plant Resources [DPR].

Isolation of pure culture of mycelium

Isolation of pure culture of mycelium was performed by tissue culture method [20]. Sterilized inner part of tissue [mycelia] from Pileus and Stipes were inoculated in sterilized Potato Dextrose Medium [PDA] slant tubes under sterile condition and incubated [BOD incubator, Optics Technology] at 25°C for 1-2 weeks. Pure culture of mycelium was obtained by repeated sub culturing process.

Grain Spawn

The protocol was followed as developed by Chang and Miles with slight modifications. 5 kg of wheat grains was washed thoroughly in clean water, soaked for one day and mixed with 7.5g of CaCO₃. The grain mixture was filled in heat resistant polypropylene bags of cover size 28 x 10 cm² and their mouths were closed using rubber bands. The bags were sterilized in autoclave at 15 lb/in² pressure at 121° C for 30-45 minutes. Pure culture of mycelium was transferred aseptically to sterilized bags filled with substrate. The bags were finally incubated [BOD incubator, Optics Technology] at 25° C for 2- 3 weeks [20].

Bed Preparation and Spawn run

Good paddy straws were selected and chopped into 3-4 inches; washed thoroughly and soaked in boiling water for 60-90 minutes and allowed to cool after draining excess water. Sterilized rice husk and saw dust was added at

concentration of 3-5% by weight of paddy straws. 60 x 30 cm² sterilized polypropylene bags were used for packaging of substrate and spawn. Spawns were inoculated at every 5 cm layers during packaging of substrate. Thus, prepared bags were incubated at 25°C in incubator [BOD incubator, Optics Technology]. The spawn run period was 8-10 days and humidity was maintained at 80-90%. Mouth of the polypropylene bags were opened for 1-2 hours once the spawn run period was over for proper air circulation under sterile condition. The incubation was done till the primordial formation.

Development of Fruiting bodies

During fruiting bodies development, the mouths of the bags were opened up to 6 hours a day under sterile condition for air circulation. Humidity and temperature were maintained up to 90% and 28 ± 2°C respectively. The fruiting bodies were harvested at egg stage and then in mature stage.

Results

Morphology of the mushroom



Figure 1: *V. bombycina* A. Hymenial surface B. Upper surface C. Spore print D. Spores [40X].

V. bombycina was observed as large pileus creamy white, dry, and covered with silky hairs measuring ~10 cm in diameter. Stipe is 13 cm long and 1-2 cm thick. The stipe was tapering upwards, cylindrical often curved, dry, white, smooth without a ring. The volva is cup-shaped with irregular margin. It measures 4 cm long, 2 cm wide, thick, white to yellowish or brownish, and mouth open sac like. Lamellae are free, at first whitish, later becoming pink, crowded, margin entire **Figure 1 A&B**]. Spore print was observed as rosy or pink in color [**Figure 1C**]. *V. bombycina* spores were elliptical, smooth, Cystidia long; variously shaped. Pileipell is

without gelatinized hyphae. Clamp connection was not seen (Figure 1D)

Pure culture of mycelium and spawn preparation



Figure 2: *V. bombycina* A. pure culture of mycelium B. Microscopic view [100X] and C. Mother spawn.

White mycelia were isolated on the PDA tubes. Septate mycelium was observed under microscopic view (Figure 2 A & B]. The mother culture of the mycelium of *V. bombycina* was initiated after 4-5 days of incubation. The pure culture of the mycelium was isolated by repeated sub culturing on PDA tubes. Incubation period varies between 15-20 days depending upon density of mycelium.

The spawn was developed in wheat grains. The mycelium was fully colonized in wheat grains after incubation of 2-3 weeks (Figure 2 C).

Fruiting bodies development



Figure 3: Cultivated fruiting bodies A. early stage B. mature stage.

Mycelium colonization was observed in substrates after one week of incubation. Primordial was initiated during 10-15 days of incubation. Button stage and mature fruiting bodies were observed during 15-20 days and 20-30 days respectively (Figure 3)

Discussion

The Genus *Volvariella*, is easily recognized with pink lamellae and rosy or pink in color spores in Figure 1 D.

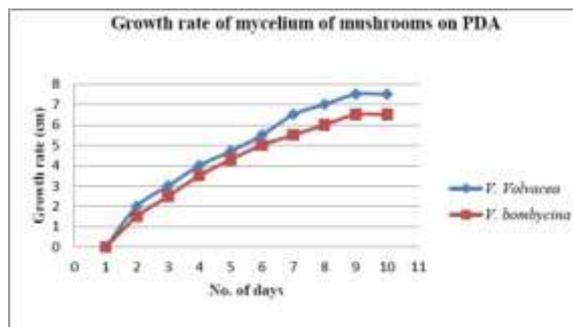


Figure 4: Showing growth curve of mycelium of *V. voluacea* and *V. bombycina* at 25°C

The stipe of the fruiting bodies doesn't have an annulus. It has volva at the base of the stipe. The lamellae of *Volvariella* species are whitish at first, which later becomes pink [6]. Growth rate of mycelium of *V. bombycina* showed slower in contrast to *V. voluacea* (Figure 4). According to Jonathan & Awotona in 2011, mycelia of *V. bombycina* showed best growth at 28° C and pH 6.8 [21].

It has been reported that straw alone is not sufficient as a composting material as it contains a little quantity nutrients and has a slow rate of decomposition [25]. The cultivation of wild edible mushroom *V. bombycina* was grown by using local agricultural waste substrate, paddy straw supplemented with saw dust and rice husk. Formulation of mushroom substrates presents a very essential factor for the multiplication of mycelium, as it allows the penetration of the mycelium, which influences the fruiting of mushroom. Mushroom produces various enzymes which makes it capable of utilizing the complex lignocellulosic organic compounds [22]. It took 1-2 weeks for complete mycelial colonization in the substrates. Incubation allows the colonization of mycelium in the substrate of the paddy straw mushroom, mycelium of *V. bombycina* grows at slightly low temperature [25°C-30° C] compared to *V. voluacea* and requires relative humidity 80-90%. A variety of waste materials have been used for cultivation of *Volvariella* mushroom that include paddy straw, water hyacinth, oil palm bunch and oil palm pericarp waste, banana waste, sawdust, cotton waste, sugar cane bagasse, composted mixtures of tropical wood wastes and pineapple skin waste. These substrates provide essential macro elements for crop

production [potassium, calcium, phosphorous, magnesium, nitrogen and sodium] [20].

The formation of fruiting primordial or initials requires minimal ventilation and light penetration to stimulate the synchronized fruiting. Formation of fruiting primordial is one of the most critical stage which depend upon physical factors viz. temperature, ventilation and light [23]. The transition from the vegetative hyphae to the formation of primordial and the subsequent development of fruiting bodies depends on many factors, genetic as well as environmental. Environmental factors can affect the yield the yield, timing of fruition and other characteristics of the crop depending upon its genetic potential [20]. Consequences between environmental factor and mushroom growth substrates have been reported to play an important role in inducing the formation of fruiting bodies [24].

Conclusion

Hence, in vitro cultivation procedure for *Volvariella bombycina* under laboratory conditions was optimized. The optimized protocol can be utilized for commercial scale cultivation with further processing and optimization.

Author's Contribution

PKC, BHP and MKA conceived and designed the experiments; PKC performed the experiments; PKC and MS analyzed the data; PKC wrote the paper; MS revised and proof read the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of Interests

The authors declare no competing interests for publication of this paper.

Ethical issues

As the research involved no human or animal specimens, ethical approval for conducting this research work was not necessitated.

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