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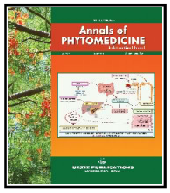

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Optimization of growth media and light requirement for obtaining maximum biomass and yield of mushroom (*Cordyceps militaris*)

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Abstract

This study is based on optimisation of maximum mycelial growth of mushroom, *Cordyceps militaris* and developed a standard protocol for high yield production of *C. militaris*. For *C. militaris,* vegetative growth and high yield production, this study parameter offers fundamental information. *C. militaries* grow in high altitude in very harsh conditions in the Himalayas. A laboratory experiment was conducted to investigate the effects of different sugar concentrations on mycelial growth in liquid spawn in combination with nutrient solution and spawn quantity for high-yield generation of *C. militaris*. The results showed that the *C. militaris* mycelium covered the surface quickly in liquid media containing dextrose sugar content @ 30 g/litre and that the maximal mycelial development was observed in the substrate with 12.5 ml of added spawn, compared to other spawn concentrations. The maximum fruiting body production was observed in 35 g of brown rice + 30 ml of nutrition solution, which also contained 12.5 ml of liquid spawn containing 30 g/l of dextrose sugar. Following observation and analysis, it was concluded that a combination of 35 g of brown rice and 30 ml of nutrient solution, along with a sugar solution @ 30 g/l, produced the maximum production and the higher primordial and fruiting bodies of *C. militaris*.

1. Introduction

The ancient Greeks, Egyptians, Romans, Chinese, and Mexican civilizations valued mushrooms as a delicacy, were aware of their therapeutic benefits, and even incorporated them into religious rituals (Chang and Miles, 1987). At least 12,000 different fungus species can be categorized as mushrooms, with at least 2000 of those species displaying varying degrees of palatability (Chang, 1999). About 300 species have been experimentally developed, and 60 have been commercially grown. Most of these domesticated species have both culinary and therapeutic qualities. Mushroom is considered as "Food of the God" and is considered as a special kind of food since ancient time. It is an achlorophyllus in nature, a fungus that grows in various environments and occurs seasonally on all continents. Mushrooms are in the limelight and evoked interest globally for their bioactive compounds that find their pharmaceutical and therapeutical values as well as their nutritive value (Gayathiri *et al*., 2021).One of the most important therapeutic mushrooms among these is the caterpillar fungus (*Cordyceps* spp.). 750 species of the genus *Cordyceps*, an entomopathogenic fungal group, have been identified (Sung, 1996). It is an entomophagous fungus, which belongs to the phylum Ascomycota. The fungus parasitizes larvae of ghost moths (Lepidoptera) or Thitarodes (*Hepialus* spp.) moths (Akshaya *et al*., 2021). The names are derived from the Latin words chord and ceps, which respectively mean "club" and "head." China refers to this as "soft gold" (Winkler, 2008). It has a wide variety of active ingredients, including cordycepin, polysaccharides, ergosterol, and mannitol, and because of its numerous physiological functions, it is being used for a variety of medical conditions (Song *et al*., 1998; Nag and Wang, 2005). The continuous supply of healthy and nutritional foods for the current huge population is a big challenge and it shall become more threat for human in the upcoming time. So, it is indispensable to look for new crops that can satisfy demand of food and nutrition (Mehrotra, 2021). Numerous studies to increase cordycepin production, have improved *C. militaris'* fruiting body and mycelial output through artificial cultivation (Masuda *et al*., 2007). An entomopathogenic fungus belonging to the Ascomycota phylum, Hypocreales order, and Clavicipitaceae family is called *C. militaris* is frequently utilized in traditional South Asian medicine and cuisine. Despite not being classified as a mushroom by taxonomy, the Ascomycetes phylum has historically been utilized as a medicinal fungus. The Latin terms "cord" and "cephalic," which are connected to "club" and "head," are whence the name "Cordate Club" originated. 450 species of the *Cordyceps* genus have been reported (Kumar *et al*., 2023)

2. Materials and Methods

2.1 Experimental site

The experiments were conducted in the Mushroom Laboratory Department Plant of Pathology, B.A. College of Agriculture Anand Agricultural University, Anand, Gujarat, India during year 2022-23.

2.2 Establishment of pure culture

The ICAR, located in Solan, Himachal Pradesh, provided the *C. militaries* strain that was employed in this investigation. The culture was maintained on a PDA medium in full darkness and stored in a

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refrigerator at 5-7°C for future research after being cultivated in sterile petri plates for 8-10 days. Single-branched hyphae from the expanding colony's perimeter were marked at low power (10x) in the compound microscope and then transferred to PDA slants for this purpose. Slants were incubated at 25 ± 2°C giving a 12/12 alternate cycle of light and darkness of near ultraviolet (NUV) for 7 days (Parmar and Gohel, 2023). These tubes were again sub-cultured on PDA after being incubated, and then they were stored for later use in a refrigerator at 5-10°C (Dlamini *et al*., 2012).

2.3 Effect of different sugar concentrations on time and spore formation of liquid spawn

One liter of distilled water should be used to prepare the liquid spawn, and six different sugar concentrations (ranging from 10 to 35 g/l) should be added. The experiment called for 10 g of yeast extract and 10 g of peptone. Each flask should be filled with 100 cc of medium before being autoclaved at 15-20 psi for 30 min. After cooling, use laminar air flow to inoculate these flasks with a pure culture of *C. militaris* that is 5-6 days old. For ten days, rotate the inoculated flask (Figure 1). The production of mycelial pieces was tracked during each treatment's 10 days incubation period in triplicates.

2.4 Effect of substrate and nutrient solution mixtures on fruiting body formation

C. militaris was grown on three different nutrient solution concentrations using brown rice as the grain substrate to examine the most advantageous substrate combinations for fruiting body production. In 1000 ml of distilled water with brown rice as the basal medium, the nutrient solution, consisting of glucose (30 g), yeast extract (3 g), peptone (5 g), KH_2PO_4 (2 g), $MgSO_4$ (0.5 g), vitamin B12 (10 mg) and multivitamin (10 mg) was created. Brown rice should be completely washed after 30 minutes of soaking in water. Put the rice in the strainer and give it 30 min to dry. In three duplicates, each of the three concentrations-M1 (30 g brown rice + 35 ml nutrient solution), M2 (25 g brown rice + 40 ml nutrient solution) and 35 g brown rice + 30 ml nutrient solution was added to the glass container. These bottles were autoclaved at 121°C for 20 min. After allowing the glass to cool, the best-growing *C. militaris* liquid spawn was carefully poured into each bottle in concentrations ranging from 2.5 to 15 ml, and the bottles were then moved into a growth chamber with a humidity of 75% and a temperature of 20°C. Regular measurements of the mycelium's growth were made.

2.5 Effect of light used on pinhead and fruiting body development

For the induction of pinhead and fruiting body development, white or pink light is typically used. However, in this study, a special LED light was used that combined pink light (1/3, 450-460 nm + 2/3 620-630 nm), blue light (450-460 nm), and red light (620-630 nm) with a total illumination of 750-800 lux and 11 watts (Figure 2). Dark incubation acts as a catalyst in the synthesis and elements of the mushroom extract act as reducing and stabilizing agent (Sneha *et al*., 2022).

3. Results

3.1 Effect of different sugar concentrations on time and spore formation of liquid spawn

In the liquid spawn medium utilized for testing, mycelium growth of *C. militaris* was detected at various glucose concentrations, *viz*.,10 g to 35 g, and it was found that the largest amount of mycelium pieces was observed at 30gm of glucose concentration after 7 days. Due to greater sugar levels in the contaminated liquid spawn, minimal growth was seen at a concentration of 35 g glucose. A suitable sugar concen-tration for the mycelium growth of *C. militaris* was found in liquid spawn medium with a concentration of 30 g at 15°C to 20°C (Figure 1).

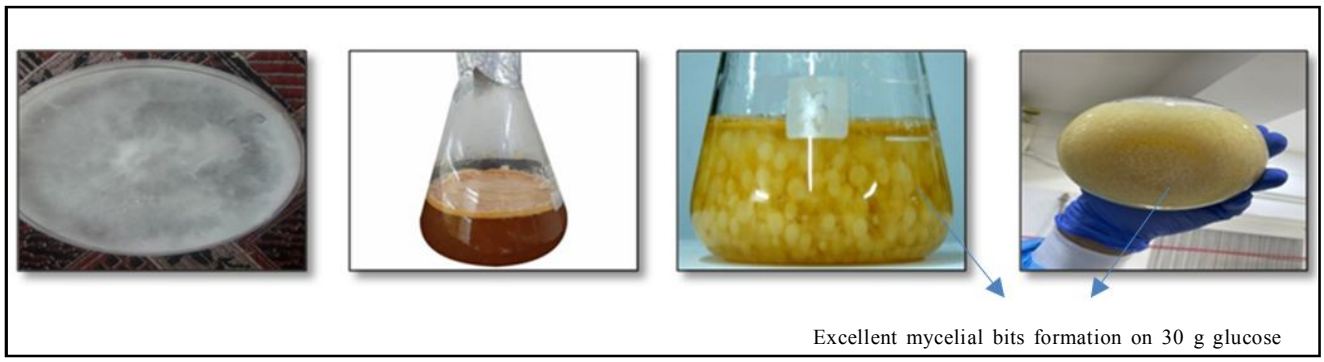

Figure 1: Process of spore formation of liquid spawn on different sugar concentration.

3.2 Effect of substrate and nutrient solution mixtures on fruiting body formation

The early liquid spawn assay revealed that the vigorous mycelial growth were produced at a glucose concentration of 30 g, which were subsequently used for spawn inoculation at various concentrations between 2.5 ml and 15.0 ml to create fruiting bodies. According to the results of the experiment, the substrate and nutrient solution concentration (35 gm of brown rice plus 30 ml of nutrient solution) covered the early mycelium in spawn concentrations of 10.0 and 12.5 ml.

3.3 Effect of light used on pinhead and fruiting body development

Normally, 800-1000 LUX pink or white light is used to induce the formation of pinheads and fruiting bodies, but in this study, we used an LED light of 11 watts with a combination of pink, blue, and red light for a total of 800 LUX for 12 h, and we discovered that pinhead formation began at 12 to 14 days in 35 g of brown rice plus 30 ml of nutrient solution and 10.0 or 12.5 ml of liquid spawn per bottle. Normally, the yield of *C. militaris* was 7-8 g per bottle, but by adjusting the liquid spawn's sugar concentration and quantity, along with the amount of brown rice and nutrient medium, it was able to produce 10-12 g per bottle (Figure 2).

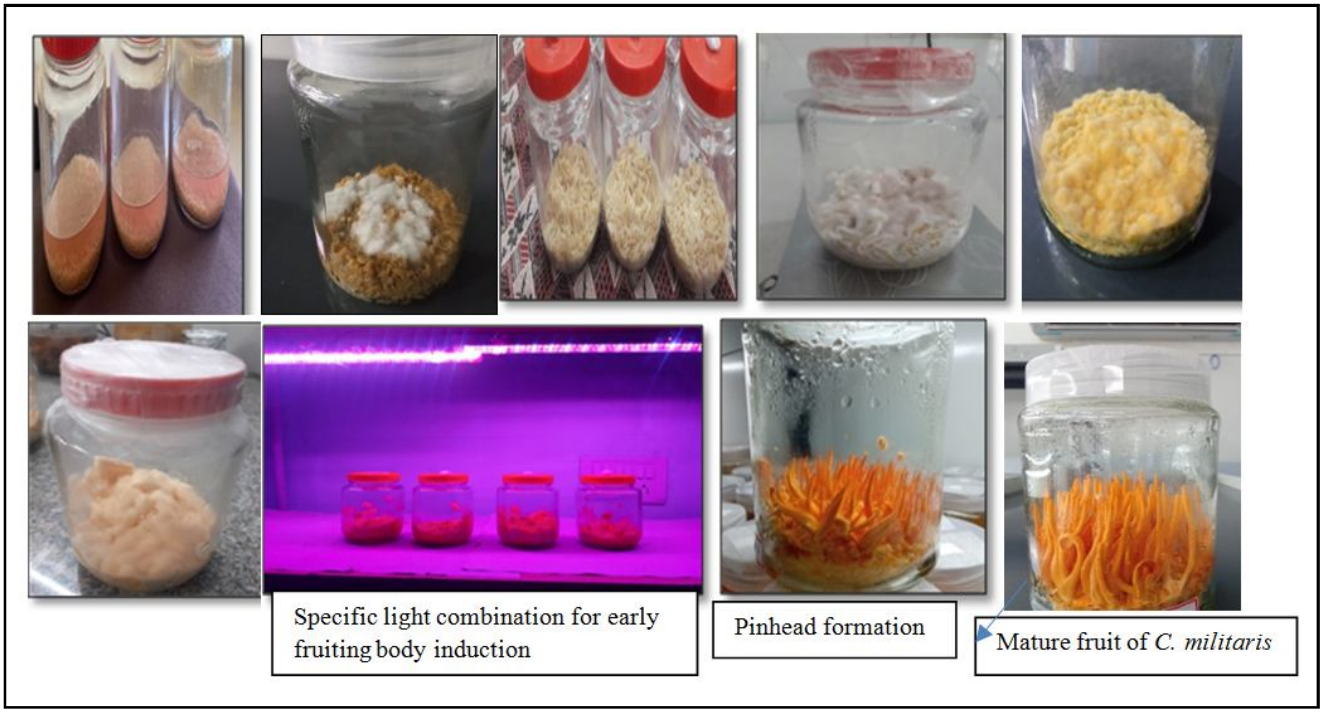

Figure 2: Fruiting body development stages of *C. militaris* on different concentration of brown rice and nutrient solution.

4. Discussion

In the present investigation, maximum mycelium growth was found with a sugar concentration of 30 g at 15°C to 20°C. and the early mycelium in spawn concentrations of 10.0 and 12.5 ml was covered by the substrate and nutrient solution concentration (35 g of brown rice plus 30 ml of nutrient solution). *C. militaris* produced 7-8 g in every bottle, but it was able to produce 10-12 g by varying the volume and sugar content of the liquid spawn, as well as the amount of brown rice and nutrient medium. The results of this study agree with those of earlier studies.

Gour *et al*. (2021) stated that in CDA (Czapek Dox Agar) nutritional media, *C. militaris* was shown to be more likely to grow. Maximum mycelium growth was recorded at 20°C, whereas optimal growth happened at pH 6. At a concentration of 20 g/l, carbon sources like glucose and dextrose accelerated the growth of *C. militaris* mycelium. Further, their study on the production of fruiting bodies in various grains, including brown rice, wheat, millet, chickpea, and maize, reveals that brown rice has the highest fruiting body production. Additionally, research on various liquid media (M1, M2, M3, M4, and M5) utilising brown rice as a grain substrate leads to the conclusion that M3 liquid media produces the highest yield, primordia, and maximum fruiting body of *C. militaris*. Yin and Qin (2009) revealed the optimal growth temperature range for *C. militaris* mycelium is 20 to 25°C. *C. militaris* has been grown at pH values of 6.0 and 7.0, respectively.

Ha *et al*. (2020) found the red : blue light combination with a wavelength ratio of 5:5 or 3:7 had the strongest influence on the generation of cordycepin among the three wavelength combinations. The ideal growth parameters were 19.2278 h of light per day, 9.19497 g of glucose per 50 milliliters of medium, and 53.112 h of cultivation time. A maximum yield of 2860.01 µg/ml cordycepin was anticipated by our model. In order to confirm the computed maximum, we conducted tests using growth media that reflected the ideal combination that we had found and the cordycepin yield of 2412.5 µg/ml.

5. Conclusion

Throughout the entire study, liquid media for spawn was adjusted (with a different sugar concentration) and monitored for the spore generation and early mycelium development. Second, it was determined that the amount of brown rice, which was 35 g of brown rice plus 30 ml of nutrient solution and 10.0 or 12.5 ml of liquid spawn each bottle, had been started at 12 to 14 days. Normally, the yield of *C. militaris* was 7-8 g per bottle, but by adjusting the liquid spawn's sugar concentration and quantity, along with the amount of brown rice and nutrient medium, it was able to produce 10-12 g per bottle.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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