

**STANDARD OPERATING PROCEDURE FOR *CORDYCEPS MILITARIS* MUSHROOM
CULTIVATION**

Materials Needed:

1. *Cordyceps militaris* culture/liquid spawn
2. Substrate (usually a mixture of rice, grains, or other suitable materials)
3. Sterilization equipment (pressure cooker or autoclave)
4. Petriplate and Glass jars containers
5. pH meter and pH adjusting solutions
6. Incubation chamber or room
7. Laminar Air flow Chamber
8. Tray dryer
9. Humidity and temperature control equipment
10. Light source (for inducing fruiting)
11. Humidifier
12. AC
13. Shaker
14. Incubator
15. Refrigerator
16. Weighing Balance
17. Clean working area

Procedure:

1. Preparation of Substrate:

- Prepare a suitable grain-based brown rice substrate

- Soak the substrate for 20 minutes in water

2. Sterilization:

- Fill the jar with substrate and media
- Cover the hole with micro filter tape for air circulation.
- Sterilize the substrate in an autoclave or pressure cooker at around 121°C (250°F) for 1-2 hours to kill competing microorganisms.
- Let the substrate cool down to room temperature before inoculation.

3. Inoculation and Culture Development:

- In a laminar flow hood, transfer *Cordyceps militaris* spores onto the prepared substrate using a sterile inoculation loop, needle, or pipette.
- Seal the jar.
- Incubate the cultures in a controlled environment at around 25-28°C (77-82°F) with high humidity (90-95%) and low light intensity for 2-4 weeks, depending on the growth rate.

4. Mycelium Expansion:

- Observe the growth of the mycelium and check for any signs of contamination.
- Once the mycelium has fully colonized the substrate, transfer a piece of the colonized mycelium to a fresh substrate in a larger container using a sterile scalpel or knife.

5. Transfer and Fruiting Initiation:

- The mycelium will reach its maximum growth potential
- Create a conducive environment for fruiting by decreasing the temperature slightly (around 18-22°C or 64-72°F) and increasing humidity to near saturation (95% or higher).
- Expose the culture to indirect light for 12 hours per day to induce photoperiodism.

6. Fruiting and Harvesting:

- Watch for the formation of fruiting bodies (ascocarps) from the mycelium. They will appear as small orange bumps that will gradually elongate and develop into caterpillar-like structures.
- Maintain high humidity and indirect light to encourage fruiting body development.
- Harvest mature fruiting bodies carefully by cutting them at the base with a sterile scissors or knife. They will be ready when they have reached their full size and color, usually after 2-3 weeks.

7. Spore Collection (Optional):

- If you want to collect spores for future use or to start new cultures, you can let some of the mature fruiting bodies release spores onto a clean surface, such as a piece of paper or glass.
- Collect spores by scraping them off with a sterile spatula or brush. Store them in a sealed container in a cool and dry place.

8. Cleaning and Maintenance:

- Regularly clean the laboratory environment and equipment with disinfectants to prevent contamination.
- Maintain aseptic techniques when handling cultures and substrates.
- Dispose of used substrates and cultures properly according to safety regulations.

9. Scaling Up (Optional):

- If you want to scale up your production, you can use larger containers or growing systems, such as bags, trays, bottles, jars, or boxes.
- You can also experiment with different substrates, temperatures, humidities, light intensities, and inoculation methods to optimize your yield and quality.

Remember that *Cordyceps militaris* cultivation can be complex and may require optimization based on your specific laboratory conditions and available resources.