

## Cordyceps Method of Cultivation, It's Importance and Health Benefits

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In recent years, mushrooms have become a major and important source with various bioactive ingredients. The edible species were called mushrooms and non-edible ones toadstools. Cordyceps is an annual Ascomycetes parasitic fungus. It is originated from Greek and Latin word Greek kordyle meaning 'club' and latin ceps meaning 'head'. The genus Cordyceps has 90 species grows mostly on insects, caterpillar fungi and vegetable caterpillars (H.C Dube, 2013). Some species grow on sclerotia of *Claviceps* and ascoma of *Elaphomyces*. It is one of the oldest entomogenous fungus reported as plant worms. The dead caterpillars having the fungal sclerotium are eaten as a delicacy and tonic in China.

Cordyceps were first used in the mountains of Tibet thousands of years ago as a remedy for fatigue and recovery from illness. Its medicinal properties are due to variety of therapeutically important constituents including cordycepin, cordyceps acid, selenium, amino acid like lysine, glutamic acid, proline, threonine and cordyceps polysaccharide. But Cordycepin (3'-deoxyadenosine) is one of the most versatile metabolites of *Cordyceps militaris* due to its broad spectrum of biological activity. *C. militaris* is erect having soldier like stromata. The infection is initiated by the filiform ascospores or by fragments landing on the insect host.

*C. militaris* and *C. sinensis* have been described as an exotic medicinal mushroom that used since ancient time to improve the immune system and boost energy levels. *C. sinensis* and *C. militaris* are recommending for all illnesses, as a tonic because it improves energy, appetite, stamina, libido, endurance, sleeping patterns, diabetes and aging (Kinjo N, Zang M. 2001). It also can reduce tumor formation in a model of metastasis in mice and has therefore been proposed as a cancer drug. The low doses of cordycepin can reduce the length of poly (A) tails and the proliferation of NIH3T3 fibroblasts. The higher doses of the cordycepin

inhibit the cell attachment and reduce focal adhesions. The fruiting bodies of this mushroom can be successfully obtained through cultivation *in vitro* in brown rice broth or brown rice solid medium. *In vitro* the fruiting bodies of *C. militaris* grow extremely slowly, which need about 30-45 days or more. It requires temperature at 18 to 22 ° C and humidity 65 to 70%. Production of cordycepin increased about 97% by liquid fermentation. *In vitro* the fungus produces cutinase enzyme.

### **In vitro cultivation methods of *C. militaris***

#### **Method of preparation of media**

For the preparation of 1 Litre agar media add peeling potato -200g, sucrose 15-20g, peptone 1-5g, egg 7-10g, agar 18-20g, Cobastab 1 0.2-0.3g, magnesium sulfate 0.2g, water 1 litre.

#### **Solid medium preparation method**

The quantity of materials takes respectively above-mentioned in proportion as required, take 500ml of water in a pot add 200g washed, peeled and sliced potato, boil 25-35 min or the time till they are easily penetrated by a glass rod, filter through cheese cloth, in supernatant, add Cobastab, sucrose, magnesium sulfate, whole juice of the egg, peptone and agar, add water and be settled to 121 °C of sterilizing in autoclave for 20-25 min after 1L packing. After sterilizing allow the flasks to cool until the flask can be held by hand.

#### **Inoculation method**

Pour melted and cooled medium into sterile Petri dishes or on the flat board. Allow the agar to solidify. Inoculate the mother caterpillar fungus into rich solid culture medium containing Petri dishes or the flat board or in containers. After inoculation incubate it at 20 °C ± 1 °C, lucifuge is cultivated 7-10 days after incubation, takes out standby when mycelia is covered with flat board.

#### **Broth medium preparation method**

For preparation of one liter broth media add peeling potato 150-200g, sucrose 15-20g, egg 3g-5g, Cobastab 1 0.2-0.3g, onion 10-15g, carrot 5-10g, peptone 1-5g, fresh *Cordyceps militaris* fruiting body 10-12g, water 1000ml. The quantity of materials takes respectively above-mentioned in proportion as required, take 500ml of water in a pot add 200g washed, peeled and sliced potato, fresh *Cordyceps militaris* fruiting body is pulverized, onion, carrot

stripping and slicing, boil 25-35min. after boiling filter through cheese cloth, in filtrate, add sucrose, peptone, Cobastab vitamin and whole juice of the egg to boil, add water and be settled to 1L. after that Sterilized in autoclave at 121 degree centigrade for 20-25min. After sterilizing allow the flasks to cool until the flask can be held by hand.

### **Inoculation method**

Inoculation are by standby solid cordyceps species access liquid culture primary surface, 20 °C ± 1 °C standing lucifuge is cultivated 48h, 100-120rpm, 18-20 °C, humidity is that the shaking table of 60%-65% is cultivated 5-7, is covered with to take out that to be placed in 4 °C of refrigerators standby after mycelia in broth nutrient medium.

### **Preparation of Cultivation base**

Take wheat and oat it is a major ingredient, each 1/3 mixing of paddy, and other auxiliary material is used dried silkworm chrysalis meal 7-10g, peptone 55-60g, sucrose 8-10g, magnesium sulfate 0.4g, potassium dihydrogen phosphate 0.4g, water 1 liter. Mix raw material of major ingredient wheat and oat and wash it with tap water, dries moisture, and packed in the Blake bottle of 500ml volume every bottle of 30g. For the preparation of auxiliary material solution add peptone, dried silkworm chrysalis meal, sucrose, magnesium sulfate, potassium di-hydrogen phosphate and water, boil it until dissolved, when it dissolve well filter it . After filtering, adds water to be settled to 1L volume. Each is equipped with in the bottle of major ingredient and add prepared auxiliary solution of 42ml and mixed well. Seal it with polypropylene film. After sealing the bottles, autoclaving at 121 °C to sterilize for 1 to 1.5 h. After sterilizing allow the medium to cool until the bottle can be held by hand, placing the sterilized medium into disinfection chamber.

### **Inoculation and picking method**

The preserving liquid spawn in 4 °C of refrigerators is placed in to disinfection chamber and recovers 4-5 h. Inoculate the liquid spawn @ 5-8ml into the each bottle and recovers the bottle. Place it in the culturing room's after sealing and temperature to be 18-20 °C, humidity be 60-65%. It can be cultivated 30-35 days, extends 12h again and enter the vernalization stage after the long saturating medium of mycelia. Vernalization is the controlled condition of culturing room 23-24 °C, 200lx white light illumination 12h, 15-16 °C, darkroom 12h, illumination and the darkroom temperature difference are 8-9 °C, humidity is 80%-95%. Take

white light illumination 12h darkroom 12h as one day so alternately, and before white light illumination every day and darkroom ventilates respectively 1 time before cultivating, each 30min. When fruit body grows 0.5-1cm complete vernalization enter urge grass the stage.

Urge grass is the hole of first pricking 3-5 1.5mm on the plastic foil of sealing is as air-vent. The controlled condition of initiation stage culturing room is illumination temperature 23-24 °C, and temperature is 18-20 °C, humidity is 85%-95%, before white light illumination every day and darkroom ventilates respectively 1 time before cultivating, each 30min, illumination is that white light illumination: 500lx irradiates 5h, 1500lx irradiates 3h, 3500lx irradiates 2h, 5000lx irradiates 1h, and 6000lx irradiates 1h, hockets successively and cultivates 10-15 days, when fruit body top grows burr, while forming ascospore, urge grass to cultivate. Ultraviolet ray are irradiated after urging grass to cultivate, remove the plastic foil on bottle, the 30W ultraviolet lamp that is 30-40cm by vertical range irradiates 90s, and then the white light with 200lx irradiates 12h every day, cultivates 1-3 days. UV-irradiation was cultivated after 1-3 days, observe in fruit body and grow burr, gathers in fruit body, 60 day of oven dry.

### Summary

*Cordyceps* originated from Greek and Latin word Greek kordyle meaning ‘club’ and latin ceps meaning ‘head’. It is endparasite on the larvae of bat moth. Mostly found in cold, grassy Himalayan Plateau. It is widely used in China as a traditional medicine, contain cordycepin, cordyceps acid, cordyceps polysaccharide and other bioactive compounds can artificially culture in laboratory in nutrient medium.