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In vitro propagation of *Cordyceps militaries* under different substrate and light condition

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Abstract: This *in vitro* propagation experiment of *Cordyceps militaries* on different salt concentration and light condition on growth parameters and fruiting bodies were examines. Under three different salt (KCl) concentration treatment (1 µM, 10 µM & 100 µM) and brown rice as substrate were used then results shows that 10 µM concentration shows best results and got maximum growth of fruiting bodies. Where in case of light treatment best yellow fruiting bodies were obtained under 9W and brown rice as substrate. *C. militaries* an interesting fungus with both biological and medical properties. It is a species of parasitic fungus that primarily infect insects and it belongs to the *Cordyceps* genus which included over 200 species. It is used as a medicinal purpose. This assessment aim, to cultivate *C. militaries in vitro* propagation under different salt and light conditions.

Key Words: *Cordyceps militaries*, Growth parameters, Species, *in vitro* propagation.

1. INTRODUCTION:

Cordyceps, a unique fungus, is a traditional Chinese medicine and used in various health benefits. And it is herbal product being popular for its potential in modern medicine. It's known for its natural energy booster, improving athletic performance because *Cordyceps* may improve oxygen consumption during exercise, for this it enhancing performance in sports, and also supporting the immune system in body and having potential helping the body fight off infections and different diseases. And it also improves sexual health so popularly known as natural or Himalayan Viagra. *Cordyceps militaries* belonging to the phylum ascomycota, class sordariomycetes, genus *Cordyceps militaries*. It is one of the famous species in the cordyceps genus (Holliday et al., 2017; Lee et al., (2020). It contains many bioactive compounds with positive health benefits including cordycepin, polysaccharides, ergosterol, and adenosine (Das et al., 2021; Hu et al., 2024; Chan et al., 2015).

It is found in temperate and humid environment in deciduous forests such as China, India, Europe (Box et al., 2015). *C. militaries* stayed known for hundreds of years to create biometabolite which are used as treatment for many diseases. This fungus naturally parasitizes insect's larvae, particularly those of the Lepidoptera order, forming characteristics orange, club-shaped fruiting bodies found in temperature regions of Asia, Europe (Trung et al., 2024; Raja et al., 2017). The increasing demand for natural health supplements and alternative medicines has led to a growing interest in the large-scale cultivation and biotechnological production of *C. militaries* compared to its more famous relative other Ophiocordyceps sinensis, which is rare and expensive due to is dependency on specific high-altitude environment and natural hosts. In vitro propagation of *C. militaries* facial and final achieved in the starting of 1980s. *C. militaries* can grow and cultivated from yellow fruiting bodies and liquid spawn with providing suitable condition such as light and dark condition (Sitara et al., 2022; De silva et al., 2012). *Cordyceps militaris* is an option to *C. Sinensis* because the qualitative and quantitative composition of biologically active material from in vitro-cultivated *C. militaris* does not vary from the satisfied of these medium in *C. sinensis* fruiting figure. The evaluation of the piece of *C. militaris* from the culture media flashed that the attention of cordycepin and polysaccharides is evolved than that in *C. sinensis* from the hereditary aura (Biswas et al., 2024; Raja et al., 2017).

The aim of this project was to obtain biological activity of *Cordyceps militaries* mushroom to provide different salt, hormone, light, and dark condition to cultivate the *C. militaris* mushroom in laboratory (Jadranko et al., 2021; Wu et al., 2016).This study is the first to identify bioactive compounds in mycelium from *in vitro* propagation technique, yellow fruiting bodies, medium obtained after cultivation, and selected formulation containing *C. militaris* (Eiamthaworm et al., 2022; Raja et al., 2017).About 250 species have been grown by *in vitro* propagation technique, 60 cultivated commercially. The majority of these cultivated mushroom species are both edible and nutraceutical potential medicinal properties. Out of these, Caterpillar fungus (*C. militaries*) is the one of major medicinal fungus (Krishna et al., 2024; Cui et al., (2015). *C. militaries* are considered healthy mushroom due to their low calorie and various beneficial bioactive compounds such as polysaccharides, cordycepin, and phenolics. *C. militaries* can be grown in cordyceps culture, grain such as brown rice, soyabean, and some nutrients supplements but in nature it usually grows on insects (Wen et al., 2019; Quy et al., 2019). *Cordyceps*, there occurs a broad of nutritionally and medicinal important components including various types of required biomolecules such as, vitamins, carbohydrates, proteins (Das et al., 2021; Tang et al., 2018).

2. MATERIAL AND METHODS

2.1 EXPERIMENT SITES

The experiments were conducted in Tissue culture Laboratory Department of Bioscience and Biotechnology, Meerut Institute of engineering and Technology, Meerut, U.P. (India) during year 2023-2025, which is situated on the N.H. 58, Delhi-Roorkee Highway, Baghat Bypass Road crossing, Meerut, Uttar Pradesh 250005.

2.2 FORMATION OF PURE CULTURE

For this project, the cultures were grown in sterilized Petri plate or culture tubes on Potato Dextrose Agar Medium (PDA) for 10-15 days. Small piece (20x20 cm) from stored growing colony were transferred to PDA culture tube or petri plate. These petri plates were incubated at 24-25℃ in dark for 8 days until the mycelium grow, and further kept in light for 8 days and then stored at 4℃ for subculture.

2.3 PREPARTION OF LIQUID SPAWN FROM PURE CULTURE

For the preparation of liquid spawn this purpose, the liquid spawn was prepared in one litre capacity wide mouthed conical flask. Different nutrients are required for preparation of liquid spawn for the growing purpose of *Cordyceps militaries*. The components such as potato starch, glucose, mineral water, peptone, yeast extract, magnesium sulphate and other minerals. This medium was autoclaved for 40minutes. After autoclaving cooled medium inoculated with small piece of above *cordyceps* pure culture with shaking in B.O.D with 28℃ for 7 days, after seven days fibres were formed in medium and used for formation of substrate.

2.4 PREPARTION OF SUBSTRATE

In this project, different types of grains were used as substrate. For this project four grains such as Sorghum, Barley, Bajra and brown rice. The liquid Spawn was prepared as explained above procedure The grains were filled up to (40.0 gm) in the conical flask in 10 replicates and sterilized at 121℃ for 40 minutes in an autoclave. The 7 days old liquid spawn culture of *Cordyceps militaries* was inoculated after cooling to room temperature with 5-7 drops of liquid spawn in individual conical flask under suitable condition. The inoculated were cultivated with brown rice as control condition at 25 ℃ in Dark condition for 7-8 days. After white mycelium were grow, the culture was continued for 50 days in light condition at 20℃. After that fruiting bodies of *C. militaries* was grow and used as for further subculture in future.

5. RESULT / DISCUSSION: In the above investigation of cultivation of *Cordyceps militaries* depends on the different salt composition and light intensity shows different results that the maximum mycelia growth 8.89 ± 0.13 cm was obtained on 9th day when 1µM KCl used as substrate, and the minimum mycelium growth 7.62 ± 0.9 cm was obtained on 8th day when 100 µM KCl used as substrate **(Table:-1)**. These phenomena were attributed to the effects of different substrate, light shown in **(Table:-2)** and essential salts that helps to the growth of *C. militaries* under suitable temperature.

The cultivation study of *C. militaries* by Lin et al., 2024 demonstrates that appropriate light also capable enhanced the fruiting body into regular growth. This Light-emitting diodes (LED), combine with different wavelengths-based study will be explore future key for higher growth and productivity of *C. militaries* cultivation (Lin et al., 2024; Krishna et al 2024). Therefore, this project involved using low power and LED light sources of different wavelengths for improve in

the photoperiod of *C. militaries* cultures to show the effects on the growth pattern, bioactive compounds content, and antioxidant activity.
Sunil et., (2016) experimentally shows that the liquid spawn is pure culture of white mycelium growing on a different substrate like as cereal, grain. Rice, soyabean and millet grains were used as solid substrates for cultivation of grain mother spawns of *Pleurotus ostreatus* (Oyster mushroom). White mycelium extension was observed. Where in our results shows best substrate for growing of *C. militaries* grain mother liquid spawns from the rice substrate being the good, followed by rice, then millet.

6. CONCLUSION *Cordyceps militaries* has long been popularly known as herbal/natural medicinal mushroom instead of synthetic one. They are considered as pharmacological and therapeutic medicinal mushroom. This *C. militaries* project study showed that Brown rice substrate with different concentration of salt treatment was performed, the suitable results were recorded under 10 µM KCl with brown rice for the cultivation of *C. militaries* under this treatment yellow fruiting bodies were obtained. which had a basic nutritional content of biomolecule compounds and favourable properties that helps to the growth of *C. militaries.*

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Author contribution : Author (AV) is performing origin work, Drafting, data collection and reviews the manuscript. SKB supervised designing the draft, critical review and proofreading of the manuscript.

Conflict of interest : The author of this study review and publications disclose that they have no conflicts of interest.

Sr no	Treatments		Substrates	Observation	Growth	Length	%Change
1	Control	No any salt	Brown rice	White Mycelium	Thirteen Days	6.34±0.12cm	0.00
2	Salts	1 µM KCl	Brown rice	White Mycelium	Nine Days	8.89 ± 0.13 cm	48.16
3		10 µM KCl	Brown rice	White Mycelium	Seven Days	7.78 ± 0.14 cm	29.67
4		100 µM KCl	Brown rice	White Mycelium	Eight Days	7.62 ± 0.9 cm	27.00
5	Light	15W	Brown rice	Yellow bodies formed	Twenty-five days	7.5± 0.32cm	25.00
6		9W	Brown rice	Yellow bodies formed	Twenty-five days	8.0± 0.21cm	33.33
7		7W	Brown rice	Yellow bodies formed	Twenty-five Days	6.5± 0.32cm	8.34

Table No 1: - Table shows C*cordyceps militaries* grow under different Substrates and light and their observation

Sr No	Light Treatments (W)	lumens	Growth	Length	%Change
1	0 W	500 lumens	30 Days	4.0 cm	0.00
2	15 W	100 lumens	25 days	7.5cm	25.00
3	9W	500 lumens	27 days	8.0cm	33.34
4	7W	700 lumens	27 days	6.5cm	8.34

Table No 2: - Table shows *Cordyceps militaries* grow under different light intensity and their Growth observation.

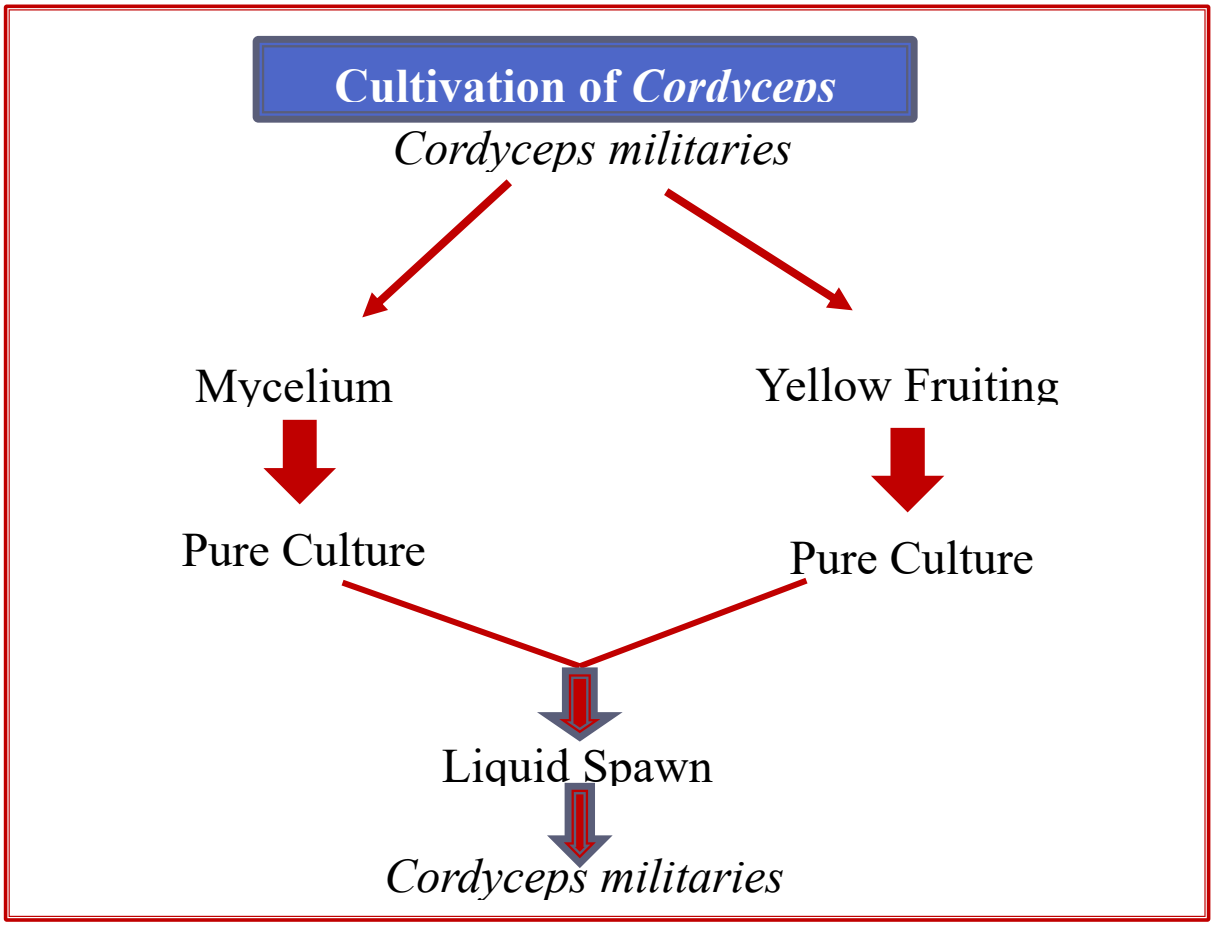

Fig: - Flow diagram show the cultivation of *C. militaries*

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