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Evaluation of different Nutrient broth and optimization of temperature for spawn production of Caterpillar Fungus (*Cordyceps militaris*)

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Abstract
Strains of *C. militaris* Indian and Japanese procured from Himalaya Cordyceps farming Noida New Delhi were used throughout the study. The fungus was maintained in Petri-plates using PDA as growth medium. Among the various liquid media tested for spawn production, the mycelial growth of Indian strain of *C. militaris* was significantly found more on JYB (0.94 g) followed by JSPYB (0.867 g. Temperature study revealed that the highest growth for Indian strain was recorded (1.670 g) at 20 days when incubated at 20 °C and least was recorded (0.750 g) after 10 days at 20 °C. However, the highest growth for Japanese strain was recorded (2.298 g) at 20 days when incubated at 20 °C and least was recorded (1.324) after 10 days at 20 °C. Mycelial growth for both of Indian and Japanese strains was ceased at 30 °C.

Keywords: *Cordyceps militaris*, mushroom, liquid spawn production, nutrient broth, temperature

Introduction
Mushroom, a fleshy and spore bearing fruiting body of fungus, is typically produced above ground, on soil, or on its food source. Mushrooms are famous for their delicacy, nutritionally rich and medicinally important sources of human food (Singh *et al.*, 2021) [8]. The world production of mushroom was 11.89 million tonnes in the year 2019. The top ten countries accounted for nearly 90 percent of world mushroom production. China was the leading producer of mushroom with 75 percent of the total world production followed by Japan, USA, Poland etc. The production of mushroom in china was 8.93 million tonnes in the year 2019. India ranked sixth in production of mushroom with 0.18 million tonnes (Priya *et al.*, 2021) [7].
Edible and medicinal mushrooms have been known for centuries and are used as functional foods and medicines in the world since 5000 BC (Panda and Swain, 2011) [5]. They are the second largest group after insects with 1.5 million fungi existing in nature (Chiu *et al.*, 2016) [1]. There are at least 12,000 species of fungi that can be considered as mushrooms with 2000 edible species. About 300 species have been grown experimentally and only 60 are cultivated commercially. Majority of these cultivated mushroom species have both edible and medicinal properties. Among them, *Cordyceps* has been historically used for the maintenance of health, prevention and treatment of various diseases worldwide (Kim *et al.*, 2010) [3]. *Cordyceps*, a kind of caterpillar-shape entomopathogenic fungus, is highly valued and is believed to be sold @ $20,000 to 40,000 kg^{-1} in the international market (Wang *et al.*, 2008; Pathania *et al.*, 2015) [9, 6]. It is the largest and most diverse genus with over 750 identified species (Olatunji *et al.*, 2018) [4].
In natural environment, both these species are parasitic of certain species of moth larvae and have similar compound profile. Although both *C. sinensis* and *C. militaris* have some similarities, they differ in their colour and appearance. *C. sinensis* grows singly from the larval head with dark brown or black stroma, cylindrical stipe and slightly swollen head (sterile apex). While as *C. militaris* arises from the buried larvae and pupae of insects with orange fruiting body which is club or clavate shaped (Xiong *et al*., 2010) [10]. However, production of liquid spawn at commercial scale has been a challenge throughout. In general, artificial cultivation methods comprise of mycelial growth at 22-25 ºC and moderate humidity of 70-75 per cent for 7-10 days. Exploring low cost liquid media for spawn production and also for increasing adoption rate of the technology at grower’s end would mean a positive effort in this regard.

Material and Methods

The present investigations on Indian & Japanese strains of *Cordyceps militaris* were conducted in division of Plant Pathology of Shere-e-Kashmir University of Agricultural Sciences and Technology, FoA, Wadura Sopore.

Evaluation of liquid media for spawn production of *Cordyceps militaris*

Three different liquid media *viz.*, Potato dextrose broth (PDB), Jaggery yolk broth (JYB) for Indian strain and Jaggery sucrose peptone yolk broth (JSPYB) for Japanese strain were evaluated for their support to mycelial growth of *C militaris* strains in comparison with a standard broth containing potato (20%) glucose (3%) yeast (0.3%) peptone (0.5%) KH_2PO_4 (0.1%) $MgSO_4.7H_2O$ (0.05%) and Vitamin B_1 0.005% (Shih *et al.*, 2010) [11]. The composition of various media as follows:

Treatment No	Media	Composition (g/l)
1	Potato dextrose broth (check)	potato 200g, dextrose 30g, yeast 3g, peptone 5g, KH_2PO_4 1g, $MgSO_4.7H_2O$ 0.5g and Vitamin B_1 50 mg
2	Jaggery Yolk broth	Jaggery 100g + Yolk 10 g
3	Jaggery sucrose peptone yolk broth	Jaggery 100g + sucrose 30g + Peptone 5 g + yolk 10 g

Optimization of temperature and for spawn production of *Cordyceps militaris*

Both the two strains *viz.*, Jaggery yolk broth (JYB) for Indian and Jaggery sucrose peptone yolk broth for Japanese strains of *C. militaris* were grown at constant temperature (20 ºC, 25 ºC and 30 ºC) in CRD with 3 replications.

Parameters was recorded:

Dry mycelium weight at 10, 15 & 20 days after inoculation.

Table 1: Effect of days, strains and temperature on dry mycelial weight of *Cordyceps militaris*

Days	Indian strain (JYB)		Sub Mean	Japanese strain (JSPYB)		Sub Mean	Days Mean	Temperature Mean
	20 °C	25 °C		20 °C	25 °C			
10	0.750	0.877	0.814	1.324	1.507	1.416	1.115^c	1.499^a
15	1.204	1.249	1.226	1.745	1.803	1.774	1.500^b	1.476^a
20	1.670	1.394	1.532	2.298	2.025	2.162	1.847^a	
Mean	1.208	1.173	1.191^b	1.789	1.778	1.784^a		
CD ($p<0.05$)	Strain (S) = 0.079 Days (D) = 0.097 Temperature (T) = 0.079 Strain*Days (S*D) = 0.137 Strain*Temperature (S*T) = 0.112 Days*Temperature (D*T) = 0.137 Strain*Days*Temperature (S*D*T) = 0.194							

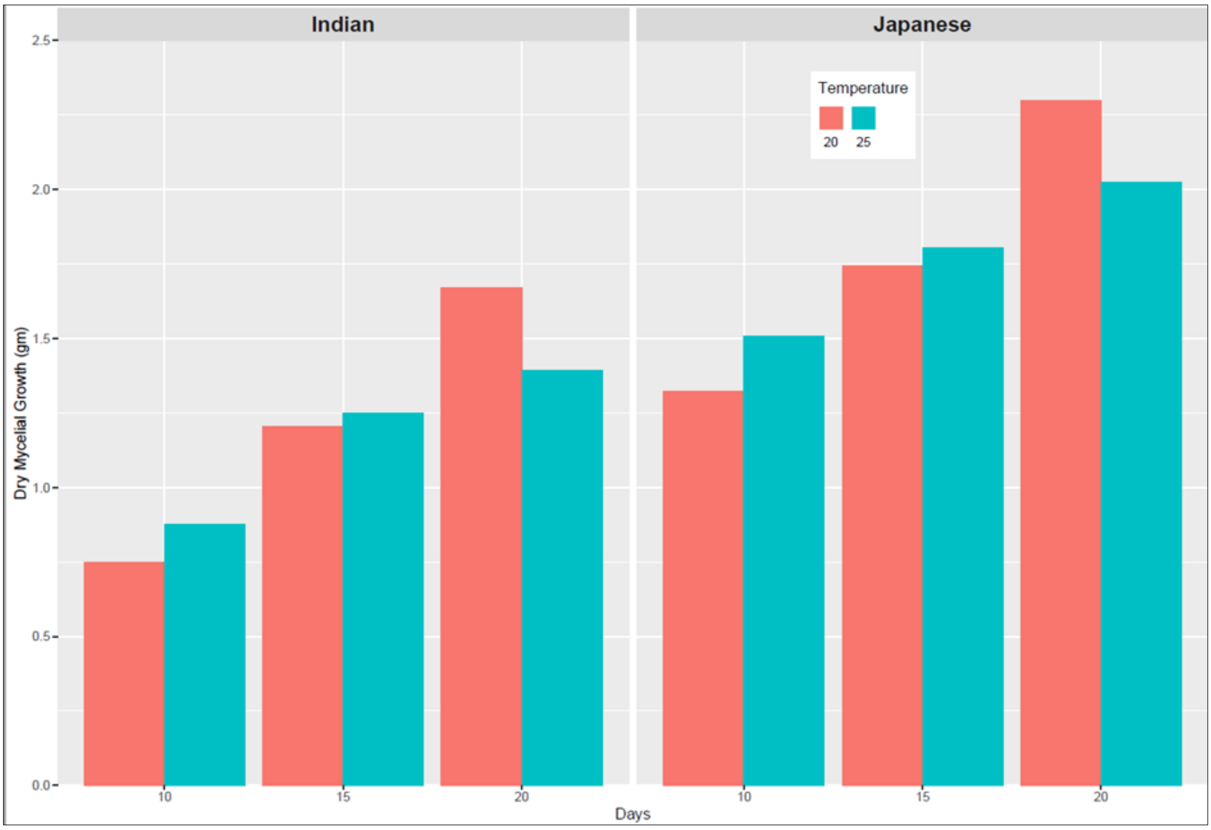

Fig 1: Graphical representation depicting effect of strains, days and temperature of *Cordyceps militaris*

Results and Discussion

Perusal of the data (Table-1) revealed that the highest growth for Indian strain was recorded (1.670 g) on 20 days when incubated at 20 ℃ followed by (1.204 g) after 15 days at 20 ℃ and least was recorded (0.750 g) after 10 days at 20 ℃. Whereas, the highest growth for Indian strain was recorded (1.394 g) after 20 days when incubated at 25 ℃ followed by (1.249 g) after 15 days at 25 ℃, while least growth was recorded (0.877 g) after 10 days at 25 ℃.

Similarly, the highest growth for Japanese strain was recorded (2.298 g) on 20 days when incubated at 20 ℃ followed by (1.745 g) after 15 days at 20 ℃ and least was recorded (1.324) after 10 days at 20 ℃. Whereas, the highest growth for Japanese strain was recorded (2.025) after 20 days when incubated at 25 ℃ followed by (1.803) after 15 days at 25 ℃, while least growth was recorded (1.507) after 10 days at 25 ℃. Mycelial growth for both of Indian and Japanese strains was ceased at 30 ℃.

The data was subjected to statistical analysis for finding out interaction between various factors *viz*., strains, days and temperature with liquid media. The analysis matrix (Table-1) indicated temperature showed non-significant interaction with temperature but significant interaction with Indian strain and non-significant with Japanese strain. Temperature also showed significant interaction at 10 and 15 days but showed non-significant relation at 20 days. Whereas, strains showed significant interactions with strains. Similar results were also observed by Hung *et al*., 2009 [2] who recorded maximum growth and cessation at 15-20 ℃ and 30 ℃ respectively, for *Cordyceps militaris.*

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Conflicts of Interest

The author declares no conflict of interest.

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