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Mycelium Performance of Medicinally Important Fungus (*Cordyceps militaris)* on Different pH and Temperature

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Medicinal Mushrooms are most valuable fungi in the world. Artificial cultivation of these fungi is trending now days in the different part of world. Many factors are influence with fungus mycelium during its growth period. So the present study was undertaken with the aim of finding out most favourable pH and temperature for mycelia growth and dry matter weight of *Cordyceps militaris*. In this study a range of pH (5.5-8.5) and temperature (16 - 28°C) was used. The Optimum pH for mycelium growth and dry matter weight of *Cordyceps militaris* was 7.0 while optimum temperature for mycelium growth and dry matter weight was 22°C.

Keywords: Cordyceps; mushroom; pH; temperature; mycelial growth; dry matter weight.

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1. INTRODUCTION

"There are at least 12,000 species of fungi that can be considered as mushrooms with at least 2000 species showing various degrees of edibility" [1]. "*Cordyceps militaris* (an entomopathogenic fungus), is one of the most important medicinal mushrooms, belonging to the class Ascomycetes, has been used popularly as a crude drug and a folk tonic food in East Asia" [2]. "The name comes from the Latin words: *cord* and *ceps*, meaning "club" and "head", respectively. It is named "soft gold" in China" [23]. "It possesses many kinds of active components (such as cordycepin, polysaccharides, ergosterol, and mannitol), and due to its several physiological activities, it is currently used for multiple medicinal purposes" [4]. "It is widely distributed in North America, South America, Europe, and Asia , from sub-tropical to temperate regions around the world". [5] "The main active constituent of Cordyceps fruiting bodies is cordycepin, which was firstextracted from *C. militaris* and then found to bepresent in *Cordyceps sinensis* [6] and *Cordyceps kyushuensis"* [7]. "Cordycepin has a broad spectrum of biological activity, plays an important role in the treatment of respiratory and cerebrovascular diseases, enhancement of body immunomodulatory function and regulation of liver and renal metabolism"[8]. Moreover, it also has been used as an anti-cancer [9], anti-tumor [10], anti-fungal [11], anti-hyperlipidemia [12], antioxidant [13], and anti-leukemia [14]. "Cordycepin is also a Phase I/II clinical stage drug candidate for the treatment of refractory Acute Lymphoblastic Leukemia (ALL) patients who express enzyme terminal deoxynucleotidyl transferase (TdT). The natural fruiting bodies of *Cordyceps* are very rare and costly to collect. Fruiting body production *in vitro* is not repeatable and cordycepin content of natural *Cordyceps* is much lower than that of culturedmycelia" [15]. "In recent years *C. militaris* is extensively cultivated in liquid as well as solid media" [16] and is "the most successfully cultivated *Cordyceps* species" [17]. Cultivation of *C. militaris* mycelium using artificial media [18] gave higher cordycepin yields. However, only a single *C. militaris* strain was employed and cordycepin production may vary with different strains.

Keeping in view the above nutritional and medicinal importance of *C. militaris* mushroom and possibilities cultivating of medicinal mushrooms in the rural as well as urban areas of the country, Present study has been done with the aim to examine the effect of different temperature and pH on *Cordyceps military,* because both are important factors which play important role in the mycelium growth as well as fruiting body production.

2. MATERIALS AND METHODS

2.1 Experimental Site

"The experiments were conducted in Mushroom Laboratory Department Plant of Pathology, S. V. P. University of Agriculture and Technology, Meerut, U.P. (India) during year 2018-20, which is situated on the Western side of the Delhi-Dehradun high way (NH-58) at distance of 10.0 km away in the north of Meerut city. The district Meerut is situated between 29^{o} 01'N latitude and 77^{o} 45'E longitude at an altitude of 237 meters above the mean sea level" [7].

2.2 Establishment of Pure Culture

"Culture of *Cordyceps militaries* were purified and maintained by single hyphal tip method. For this purpose, the cultures were grown in sterilized Petri plate on Potato Dextrose Agar Medium (PDA) for 8-10 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10x) in the compound microscope and transferred to PDA slants. These tubes were incubated at 21-24^{0}C for about a week, again sub cultured on PDA and then stored in a refrigerator at 4-8^{0}C for further use" [19]. Optimizations of Culture Conditions on redial Growth of *Cordyceps militaris*given below:

2.3 Effect of Different pH on Mycelial Growth and Dry Matter Weight

For the studies of suitable pH, the culture of *Cordyceps militaris* were incubated at seven different pH media viz. 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. Required pH of the culture media (PDA) was adjusted with N/10 solutions of NaOH or HCl, used before adding of agar, it was measured by a digital pH meter. After sterilization at 121^{0}C (1.1 kg/cm^{2} pressure) for 20 minutes in autoclave, the test media (PDA) were poured into the Petri plates (90 mm 20 ml/plate). The plates were inoculated centrally with a 9 mm diameter disc of 7 days old culture of*Cordyceps militaris*and incubated at 23±2^{0}C in B.O.D with three replications of each treatment. The observations of radial growth were taken at each 48 hrs in *Cordyceps militaris*until the colony covered the full plate.The PD broth media was poured into 150 ml conical flasks @ 50 ml per flask and after adjusting of pH, broth sterilized in

autoclave at 121^{0}C (1.1 kg/cm^2 pressure) for 20 minutes and allowed to cool. The 9 mm diameter disc cut by cork borer from the periphery of 7 days old culture of*Cordyceps militaris* were inoculated in the flask with the help of sterilized inoculation needle. The flasks were incubated at 23±2^{0}C in BOD with three replications of each treatment. "The culture was then filtered with Whatman filter paper No. 1 and the mycelium was dried at 60^{0}C in oven for 48 hours before measuring the dry weight of mycelial mat, on an electronic balance. The dry weight of mycelial mat was recorded after 10 days of inoculation" [20].

2.4 Effect of Different Temperature on Mycelial Growth and Dry Matter Weight

For studies of suitable temperature, the culture of *Cordyceps militaris* were incubated at seven different temperature viz. 16, 18, 20, 22, 24, 26 and 28^{0}C in BOD. Petri plates containing 20 ml of sterilized PDA medium were inoculated at the centre with 9 mm diameter disc of 7 days old actively growing culture of *Cordyceps militaris* under aseptic conditions. Inoculated plates were incubated in different B.O.D and three replications for each treatment were maintained. Observations on radial growth were taken at each 48 hrs in *Cordyceps militaris*until the colony covered the first full plate.

The PD broth was poured into 150 ml conical flasks @ 50 ml per flask and sterilized in autoclave at 121^{0}C (1.1 kg/cm^2 pressure) for 20 minutes and allowed to cool. The 9 mm diameter disc was cut by cork borer from the periphery of 7 days old culture of *Cordyceps militaris* and inoculated in the flask with the help of inoculation needle. The flasks were incubated at seven different temperature viz. 16, 18, 20, 22, 24, 26 and 28^{0}C in different B.O.D with three replications of each treatment. "The culture was then filtered with Whatman filter paper No. 1 and the mycelium was dried at 60^{0}C in oven for 48 hours before measuring the dry weight of mycelium mat, on an electronic balance . The dry weight of mycelial mat was recorded after 10 days" [21].

2.5 Statistical Analysis

"The suitable statistical design (CRD) was applied and the data thus obtained were analyzed statistically. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at five percent level of significance for comparison with other treatment" [22,20].

3. RESULTS AND DISCUSSION

3.1 Effect of Different pH on Mycelial Growth and Dry Matter Weight

Experiment was conducted to find out the effect of range of pH (5.5-8.5) on the mycelial growth and dry matter weight ofCaterpillar Fungus (*Cordyceps militaris*).Observations were recorded on 2nd, 4th and 6th day. The Results revealed that on 6th day, maximum mycelial growth (89.00 mm) with (14.83 mm/day) growth rate was found at pH 7.0 which was significantly superior than all other treatments while it was followed by pH 7.5 (84.00 mm) with (13.83 mm/day) growth rate. Minimum mycelial growth (62.33 mm) was recorded at pH 8.5 with (10.38 mm/day) growth rate which was significantly lower than all other treatments. It was followed by pH 5.5 (66.66 mm) with (11.11 mm/day) growth rate as shown in Table-1, Fig. 1 and Plate-1. While maximum dry matter weight (3.55 mg/50ml) of Caterpillar Fungus was observed at pH 7.0 with (0.35 mg/day)dry matter growth rate which was significantly superior to all other treatments and followed by pH 7.5 (2.54 mg/50ml) dry matter weight with (0.25 mg/day)dry matter growth rate. Minimum dry matter weight (0.93 mg/50ml) was observed at pH 8.5 with (0.09 mg/day)dry matter growth rate which was statically lower than all other treatments. It was followed by pH 5.5 (1.50 mg/50ml) dry matter weight with (0.15mg/day)dry matter growth rate as shown in Table-1, Fig. 1 and Plate-2.

The results were in accordance with the findings of Sehgal and Sagar [23] studied the effect of different pH on the growth of *Cordyceps militaris*, the best solid medium was adjusted at different pH levels, viz. 4.0 to 10.0 and observed maximum mycelial growth was observed at pH 7.5.Lee et al*.* [24] also studied the influence of pH on *Cordyceps cardinalis* and observed that mycelial growth was highest when the pH of liquid medium was 7.0 and lowest at 4.0.Pathania et al*.* [25] tested "the effect of different pH on the growth of *Cordyceps militaris*, the best solid medium, was adjusted at different pH levels (4.0 to 10.0). The best liquid medium was also adjusted at different pH levels (4.0 - 8.5). The inoculated petri plates and flasks were incubated for 10 days. The best mycelial growth of *Cordyceps militaris* was observed at pH 7.5 in solid and liquid medium respectively".

Table 1. Effect of different pH on mycelial growth (mm) and dry matter weight (mg) of *Cordyceps militaris*

pH	Radial Growth (mm)			6^{th} days Growth rate (mm/day)	Dry Matter weight (mg/50ml)	Dry Matter Growth rate (mg/day)
	2^{nd}day	4^{th} day	6^{th} day			
5.5	31.00	54.66	66.66	11.11	1.50	0.15
6.0	31.00	56.66	67.33	11.22	1.53	0.15
6.5	33.66	64.33	79.33	13.22	2.10	0.21
7.0	34.66	68.66	89.00	14.83	3.55	0.35
7.5	33.66	65.66	84.00	13.83	2.54	0.25
8.0	32.00	62.00	72.33	12.05	1.88	0.18
8.5	30.33	53.33	62.33	10.38	0.93	0.09
CD at 5 %	2.25	4.33	4.25	-	0.41	-
SE(m)	0.73	1.57	1.90	-	0.10	-

**Average of three replications*

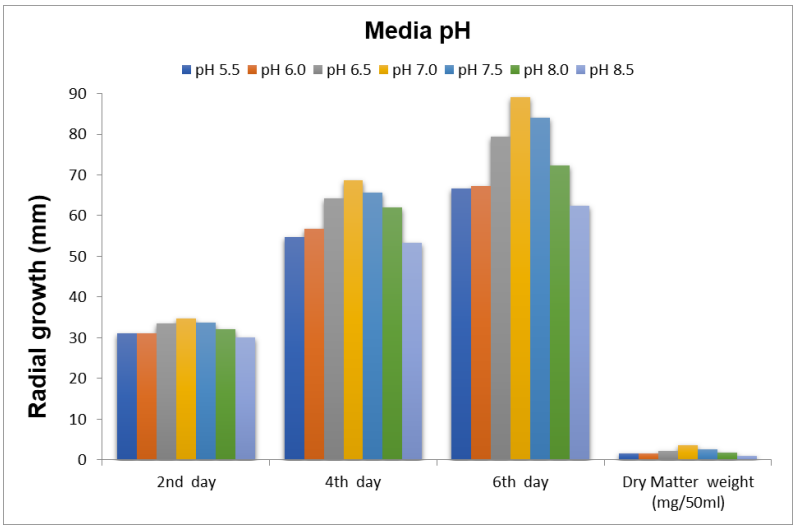

Fig. 1. Effect of different pH on mycelial growth (mm) and dry matter weight (mg) of *Cordyceps militaris*

Plate 1. Effect of different pH on mycelial growth (mm) of *Cordyceps militaris.* 1. pH 5.5, 2. pH 6.0, 3. pH 6.5, 4. pH 7.0, 5. pH 7.5, 6. pH 8.0, 7. pH 8.5

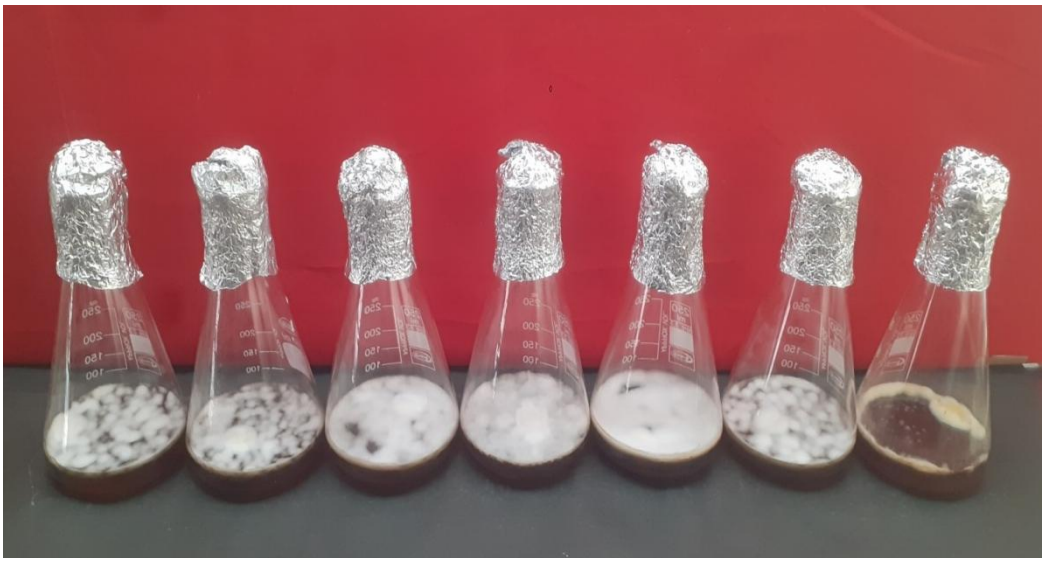

Plate 2. Effect of different pH on dry matter weight (mg) of *Cordyceps militaris*. 1. pH 5.5, 2. pH 6.0, 3. pH 6.5, 4. pH 7.0, 5. pH 7.5, 6. pH 8.0, 7. pH 8.5

3.2 Effect of Different Temperature on Mycelial Growth and Dry Matter Weight

A range of temperature (16-28 ^{0}C) was used to know its effect on the mycelial growth and dry matter weight ofCaterpillar Fungus (*Cordyceps militaris*). Observations of effect of different temperature were recorded on 2nd, 4th and 6th day and shown in Table-2, Fig. 2 and Plate-3. The Results revealed that on 6th day, maximum mycelial growth (89.66 mm) with (14.94 mm/day) growth rate was found at temperature 22 ^{0}C which was statically higher than all other treatments while it was followed by temperature 24 ^{0}C (84.00 mm) with (14.00 mm/day) growth rate. Minimum mycelial growth (52.00mm) was recorded at temperature 28 ^{0}C with (8.67 mm/day) growth rate which was significantly lower than all other treatments. It was followed by temperature 16 ^{0}C (65.66 mm) with (10.94 mm/day) growth rate.While maximum dry matter weight (1.46 mg/50ml) of Caterpillar Fungus (*Cordyceps militaris*) was also observed at temperature 22 ^{0}C with (0.14 mg/day)dry matter growth rate which was statically at par with temperature 20 ^{0}C (1.40mg/50ml) with (0.14 mg/day)dry matter growth rate and followed by temperature 24 ^{0}C (1.38mg/50ml) with (0.13 mg/day)dry matter growth rate. Minimum dry matter weight (1.18 mg/50ml) was observed at temperature 28 ^{0}C with (0.11 mg/day)dry matter growth rate which was statically at par with temperature 16 ^{0}C (1.27mg/50ml) with (0.12 mg/day)dry matter growth rate and followed by temperature 18 ^{0}C (1.35mg/50ml) with (0.13 mg/day)dry matter growth rate. The data are presented in Table-2, Fig. 2 and Plate-4.

Table 2. Effect of Different Temperature on Mycelial Growth (mm) of *Cordyceps militaris*

Temperature (^{0}C)	Radial Growth (mm)			6th days Growth rate (mm/day)	Dry Matter weight (mg/50ml)	Dry Matter Growth rate (mg/day)
	2nd day	4th day	6th day			
16	25.33	41.00	65.66	10.94	1.27	0.12
18	26.33	56.33	73.00	12.16	1.35	0.13
20	30.00	60.66	80.00	13.33	1.40	0.14
22	33.33	64.00	89.66	14.94	1.46	0.14
24	30.66	62.33	84.00	14.00	1.38	0.13
26	9.00	58.33	76.66	12.77	1.36	0.13
28	21.00	39.00	52.00	8.67	1.18	0.11
CD at 5 %	2.53	3.80	3.80	-	0.16	-
SE(m)	0.82	1.24	1.24	-	0.03	-

**Average of three replications*

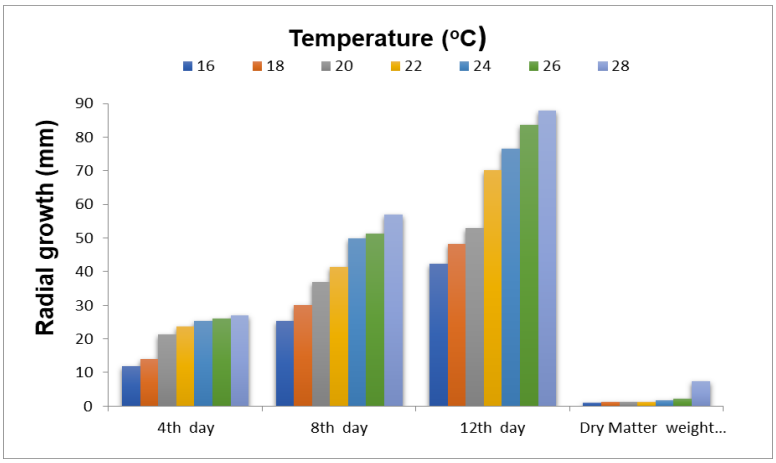

Fig. 2. Effect of Different Temperature on Mycelial Growth (mm) and Dry Matter Weight (mg) of *Cordyceps militaris*

Plate 3. Effect of different temperature on dry matter weight (mg) of *Cordyceps militaris.* 1. 16 ^{0}C, 2. 18 ^{0}C, 3. 20 ^{0}C, 4. 22 ^{0}C, 5. 24 ^{0}C, 6. 26 ^{0}C, 7. 28 ^{0}C

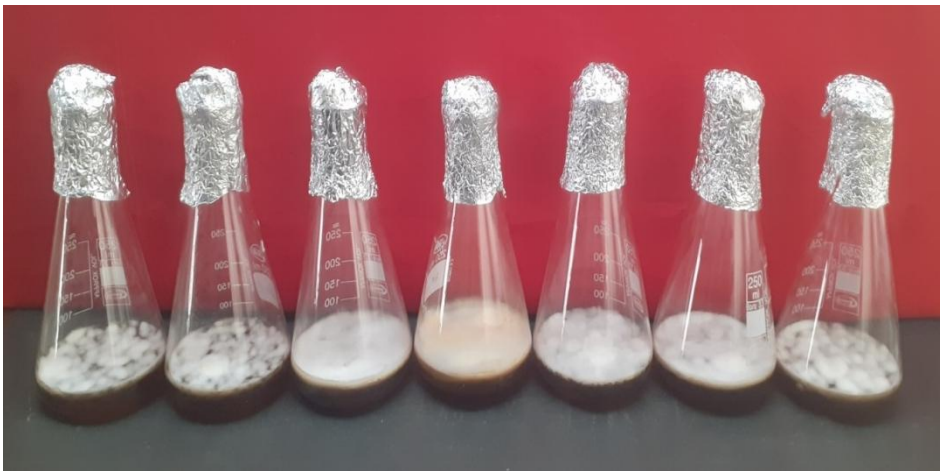

Plate 4. Effect of different temperature on mycelial growth (mm) of *Cordyceps militaris.* 1. 16 ^{0}C, 2. 18 ^{0}C, 3. 20 ^{0}C, 4. 22 ^{0}C, 5. 24 ^{0}C, 6. 26 ^{0}C, 7. 28 ^{0}C

The results were in accordance with the findings of Hung, et al.[26] reported that, the temperature from 15 °C to 20 °C was suitable for mycelium growth of *Cordyceps militaris*. Pathania et al.[25]tested a range of temperature viz. 5 to 40ºC for growth of the fungus *Cordyceps militaris* in solid and liquid medium. They found best mycelial growth of fungus *Cordyceps militaris* at 25ºC both in solid and liquid media. Adnan et al. [21] also evaluated "the optimum temperature for cordycepin production, fermentation was carried out in 250 mL conical flasks containing 100 mL of basal medium with initial pH 5.5 at 5 °C intervals in the range of 10–40°C for 20 days under static condition, and found that 25 ºC was the suitable temperature for maximum production of cordycepin".

4. CONCLUSION

Thus it can be concluded that maximal mycelium growth and dry matter weight of *Cordyceps militaris*can be achieved by cultivating fungus at pH 7.0 and in case temperature maximal mycelium growth and dry matter weight obtained at of 22^{o}C and thus it's recommended for *Cordyceps militaris* culture conditions to be use.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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