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Full Length Research Article

Mycelial Growth and Pigmentation of *Cordyceps militaris* and its Visual Correlation with Metabolites Grown on Different Culture Media

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Abstract

A prefatory experiment was conducted to examine the growth performance of *Cordyceps militaris* mushroom cultures and pigmentation using different synthetic media. The study is mainly focused on the different media, such as Saboraud's Dextrose Agar Medium (SDA), Potato Dextrose Agar medium (PDA), Vogel's agar medium, and Czapek Dox Agar medium on the growth and the pigment development in dark and light conditions of mycelium of the mushroom. Studies revealed that the average maximum growth was obtained on Vogel's agar medium than SDA medium, least in Czapek Dox Agar, followed by PDA, including extensive pigmentation. This study tries to correlate the production of pigments under different culture media and the metabolites associated with the particular colour of pigment. We intend to establish low-cost preliminary techniques to detect the presence of the major compound in the fungus with its signature colour.

***Key words*:** Pigmentation, Signature colour, metabolites, Mycelium growth, Cordyceps

Mushrooms or macro-fungi are one such repertoire that offers limitless options for medicinal, therapeutics, pharmaceuticals and nutraceuticals drug discoveries due to their ability to produce diverse beneficial secondary metabolites, proteins, polyphenols, flavonoids, polysaccharides, vitamins and minerals [1]. As a general term, Cordyceps describes a group of ascomycetous fungi growing on arthropods [2]. Cordyceps mushroom is, commonly known as caterpillar mushroom, highly medicinal and is a potent ingredient of Chinese traditional medicine (CTM); due to its positive impacts on multi diseases on human health, it has become the subject of medical research [3]. It gains worldwide recognition for the active compound cordycepin, which is an analogue to adenosine [4]. *Cordyceps militaris* is a potential harbour of bio-metabolites, boosting the present herbal treatments and gearing up the green pharmacy revolution [5]. *Cordyceps militaris* attracted the attention of de Bary more than a hundred years ago, who studied its mycelial growth by infecting larvae of *Sphinx euphorbiae* with ascospores of the fungus and growing them in moist sand [6]. Many experiments have been performed to obtain the optimal culture conditions with various factors like pH, temperature, incubation time, agitation rate, aeration and nutritional variables like N sources, C sources, N/C ratio, inorganic salts, and amino acids [5-7], []9-11]. The types of ingredients used in the media play a significant role in a mushroom culture, particularly the entomopathogenic fungi; hence the media listed above lack insoluble materials; rather, it is rich in simple sugars and inorganic salts. The insoluble fraction, which includes lignin, cellulose, hemicelluloses, protein and microbial biomass, is preferentially used by the mushroom mycelium for growth. In the present study, a stock culture of efficiently growing mycelia of *Cordyceps militaris* was cultivated on different media, i.e., PDA, SDA, Vogel's, and Czapek dox and their mycelial growth rate was determined along with the pigmentation development. The present study was indented to establish the most suitable media for the growth of mushrooms and the relation of pigmentation with the relative correlation with its major phytochemicals. This study provides insight into evaluating the *Cordyceps militaris* quality and its market potentials based on the phenology of the fruiting bodies and the color of the powdered products.

MATERIALS AND METHODS

Collection and maintenance of Cordyceps militaris culture

The pure culture of *Cordyceps militaris* was obtained from the Omcar India Pvt Ltd, Gwalior, India (a certified laboratory) and was used for mass culture production.

Inoculation and incubation

All the media were sterilized in an autoclave 15 lb/sq inch pressure for one hour and then poured into 90mm Petri dishes under the laminar flow hood to avoid contamination. Media were cooled at 37 °C. The multiplied pure culture, i.e.,

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2nd generation, was inoculated on culture media. Radial growth of mycelium was observed until the Petri dishes were filled with it. The experiment was repeated for three times. The plates were incubated at 25 °C and observed for 15 days, during which the mycelia vegetative growth and mycelial density were recorded and rated accordingly.

The mycelial density was rated as described by Kadiri [12] as follows:

+ = very scanty mycelial density
2+ = scanty mycelial density
3+=moderate mycelial density
4+=abundant mycelial density
5+=very abundant mycelial density

Measurement of growth rate

Growth rate = colony diameter on the last day (cm) / number of days measurement was taken after inoculation

Daily mycelial growth was determined using a ruler across the petri-dish horizontally.

Table 1 Preparation of different media in one litre

Ingredients in gm or ml	PDA	SDA	Vogel's agar medium	Czapek dox agar
Dextrose	20	40	-	
Agar - agar	15	15	15	
Potato Starch	4	-	-	
Peptone	-	10	-	
Vogel's salts	-	-	20	
Sucrose	-	-	15	30
Sodium nitrate	-	-	-	2
Dipotassium phosphate	-	-	-	1
Magnesium sulphate	-	-	-	0.5
Ferrous sulphate	-	-	-	0.01

All four media were prepared according to the composition mentioned above

RESULTS AND DISCUSSION

The result in (Table 2-3) on the 20thday showed that *Cordyceps militaris* had the highest mycelial colony diameter of 5.7cm, density of 5+ (very abundant) and growth rate of 0.28 on Vogel's media, followed by SDA media with 5.4 cm, 4+ (abundant) and growth rate 0.27 being colony diameter, mycelial density and growth rate respectively. On Czapek dox agar media colony diameter was 4.5cm, mycelial density 3+ (moderate), and the growth rate was 0.22. Furthermore, the least growth was recorded on PDA media which was 2.2 as colony diameter, 2+ (scanty) as mycelial density and 0.11 as growth rate. Among the four solid-growth media, Vogel's media was found to support the fastidious growth of mushroom cultures with the presence of a higher amount of inorganic salts and trace elements; this is followed by SDA, Czapek dox agar and PDA.

Light sensing plays an important role in the pigmentation of the mycelium under dark and light condition observed; (Fig 1) present that in dark condition, mycelium does not seem to produce any pigmentation, be it culture on any medium. Hence it has been inferred that light sensing plays an important role in pigmentation and the synthesis of metabolites. (Fig 2) shows the plates in light conditions where it can visualize the pigmentation initiated due to the light. Many studies on *Cordyceps militaris* under different wavelength has a holistic approach to phenology and secondary metabolism [13-14].

Fig 1 Mycelial growth in different media under dark conditions; A- PDA, B- SDA, C- Vogel's, D- Czapek dox agar

Fig 2 Effect of different media in light on pigmentation with colour ladder; A- PDA, B- SDA, C- Vogel's, D- Czapek dox agar

Table 2 Mycelial density and growth rate of *Cordyceps militaris* in a different medium

S. No.	Medium	Average mycelial density on the 15th day of incubation	Growth rate cm/day
1	Potato dextrose agar	2+	0.11
2	Saboraud's dextrose agar	4+	0.27
3	Vogel's agar	5+	0.28
4	Czapek dox agar	3+	0.22

2+= scanty, 3+= moderate, 4+= abundant, 5+= very abundant

Table 3 Mycelial growth of *Cordyceps militaris* in the different medium

S. No.	Medium	Colony diameter on different days (in cm)							
		Day 06	Day 08	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20
1	PDA	0.3	0.6	0.8	1.2	1.5	2.1	2.2	2.2
2	SDA	0.6	1.1	2.2	2.9	3.5	4.2	4.8	5.4
3	Vogel's	0.3	1.3	2.4	3.1	3.9	4.5	5.2	5.7
4	Czapek dox agar	0.5	0.9	1.8	2.6	3.1	3.6	4	4.5

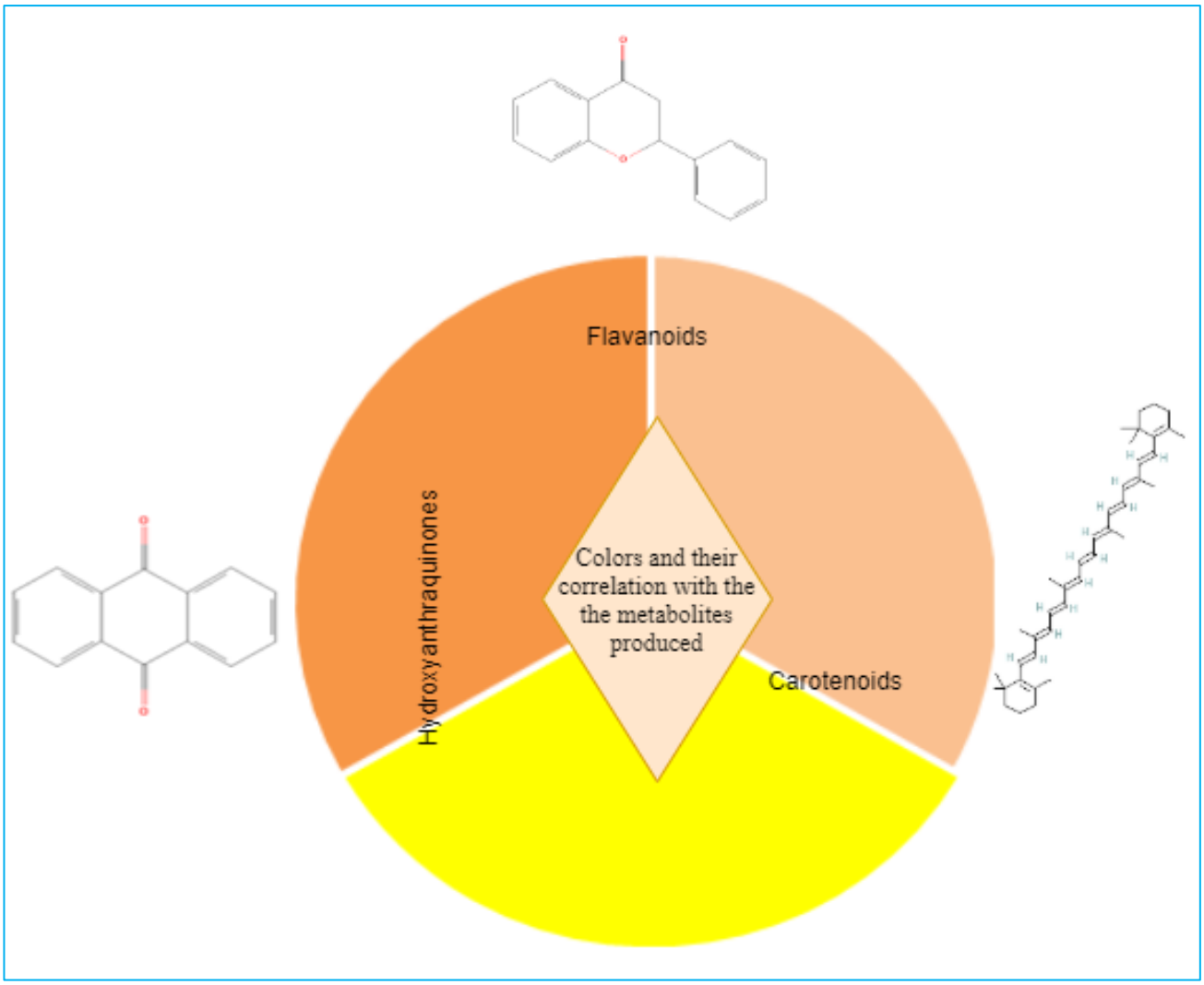

Fig 3 Colors shows the corresponding metabolites; it has been established that every metabolite has characteristic

Fungi produce different pigments like orange, yellow, black, green, blue, etc. However, *Cordyceps militaris* shows different types of pigmentation, such as deep orange or yellow, depending upon media and culture age [6], [13], [15-16]. Molecular investigation in many studies established the fact that the pigmentation depends on the type of strain; for instance, the white and yellow mutants of *Aspergillus nidulans* in contrast to the dark green of the wild forms, and the albino mutants of *Neurospora crassa* in contrast to the typical orange [15], [17]. As mentioned, *Cordyceps militaris* produces many bioactive metabolites, including ergothioneine, ergosterol, adenosine, polysaccharides, and cordycepin [4]. Among them, cordycepin exhibits a large range of health benefits. Pigmentation of fruiting bodies and mycelium gives the signature of the metabolites that the fungi have produced; (Fig 3) depicts such correlation between pigmentation colour and the major group of compounds that may present in *Cordyceps militaris*.

Wen *et al*. [13] have mentioned the effect of phenotypes on the fruiting body and its secondary metabolism of *Cordyceps militaris*, fruiting body formation and quantities of adenine, adenosine and cordycepin, polyphenols, flavonoids, carotenoids differed significantly between the phenotypes, and they reported that cordycepin production generally decreased in orange chrome to apricot orange and white colonies. This information on *Cordyceps militaris* could be used for screening high-yield strains in cordycepin production. Therefore, colony colour could be of the markers for detecting fruiting bodies and cordycepin production in *Cordyceps militaris.* Hence, the strategies for screening high-yield strains could be widely applied in other fungal biotechnology.

CONCLUSION

Since the fungal metabolites have some characteristic colour, which correlates the type of metabolites, we investigate the mycelial growth and pigmentation of *Cordyceps militaris* on different culture media to check the possible metabolites present in the *Cordyceps militaris*. This mushroom has high market value because of the presence of cordycepin (the highly demandable metabolites of *C. Militaris*). Hence the content of

the cordycepin determines the quality and price of the *C. militaris*. The study put emphasis on quick visual assessment of pigmentation on culture plate and its correlations with metabolites.

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