INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0116/2022/28–2–67–74
DOI: 10.17957/IJAB/15.1953
http://www.fspublishers.org

Full Length Article

Optimization and Effect of Different Grain Sources on Production of Liquid Spawn and Fruiting Body of *Cordyceps militaris*

Uzma Sitara[1*], Parwaiz Ahmed Baloch[1], Atta Ullah Khan Pathan[1] and Muhammad Ismael Bhatti[2]
[1]*Food Quality & Safety Research Institute, Southern Zone Agricultural Research Centre, Karachi-75270, Pakistan*
[2]*Botanical Science Division, Pakistan Museum of National History Islamabad, Pakistan*
[*]For correspondence: uzmasitara@yahoo.com
Received 15 March 2022; Accepted 20 July 2022; Published 25 August 2022

Abstract

Cordyceps militaris is an expensive and edible mushroom that is being used for therapeutic and medicinal purposes for a long time. This study was carried out to investigate the mycelial growth of *C. militaris* in a liquid medium for spawn production and fruiting body cultivation on three substrates under different growing conditions. The maximum, thick and the highest diameter of mycelial growth of liquid spawn was recorded in PD (potato dextrose) containing antibiotic streptomycin with vitamins B_6 and B_{12} at 25 ± 1°C (relative humidity 70–75%) compared to the other three medium. Artificial cultivation of solid culture medium of soaked and unsoaked sorghum, wheat and rice for the growth of *C. militaris* was also optimized in this experiment. Among all the substrates, soaked brown rice substrate was found the best treatment with the highest mycelial growth, pinhead emergence, early fruiting development had maximum weight and length in glass container procedure wrapped with polythene sheet at 23 ± 2°C (RH, 80–85%). This is the first report in Pakistan indicated that the modified plastic sheet glass jars could be efficiently and economically used as a culturing container for *C. militaris* cultivation and a substitute for expensive commercial growth containers.

Keywords: *Cordyceps militaris*; Glass container; Liquid spawn; Solid substrates; Soaked and unsoaked grains

Introduction

Cordyceps military belongs to Ascomycota. It is cultivated on a massive scale for commercial production all over the world (Mehra *et al*. 2017). Its fruiting body contains various bioactive metabolites such as ergosterol, adenosine, exopolysaccharide and mannitol (Tatani *et al*. 2016; Raethong *et al*. 2018). Cordycepin is the most metabolically active of these compounds and it has recently attracted a lot of attention as anticancer, antiasthmatic, bacteriostasis, antibacterial, anti-fungal, antiasthmatic and antihyperuricemic (Shi *et al*. 2020; Das *et al*. 2021; Li *et al*. 2021; Schwenzer *et al*. 2021). The percentage of cordycepin and bioactive components varies depending on the species, the incubation and drying conditions and the chemical structure of the fungal biomass (Rozsa *et al*. 2019). *Cordyceps* species are well-known for their high concentrations of phytochemical components, metabolites and biological properties (Zhong *et al*. 2020). It is currently also marketed as a food and beverage supplement considering the high option pharmacological advantages. For years, it has been widely utilized as a traditional food stimulant and analeptic and its therapeutic characteristics have drawn great attention (Yang *et al*. 2014). Various techniques for the synthesis of fungal biomass, both on a solid substrate and in liquid surface cultures or submerged cultures, have been developed and optimized in recent years (Rozsa *et al*. 2017; Kontogiannatos *et al*. 2021; Berovic *et al*. 2022). It is evident from earlier reports that various types of additives are incorporated in the solid media such as *Sesbania* leaves, rice, brown rice, millet, rye, bean powder, cottonseed hulls, corn grains, corn cobs, sorghum, wheat, sunflower and brewery grains (Bajwa *et al*. 1999; Chen *et al*. 2011; Shrestha *et al*. 2012; Wen *et al*. 2014). According to researchers, various cultures exhibited amazing impacts on the yield of mycelium and metabolic compounds of *C. militaris* (Panda and Swain 2011; Yi *et al*. 2014). Cereals have been commonly used in the large-scale production of *C. militaris*. However, besides cereal grains, some other substrates for the cultivation of *C. militaris* have also been studied. Lin *et al*. (2017) investigated the ability of *C. militaris* to develop on various agro-waste materials. Cottonseed shells, corn cob particles, Italian poplar sawdust and substrates spent by *Flammulina velutipes* were employed to cultivate *C. militaris* which exhibited greater suitability for culturing. However, for cordycepin production, *C. militaris* has been commercially cultivated employing solid-state and submerged

To cite this paper: Sitara U, PA Baloch, AUK Pathan and MI Bhatti (2022). Optimization and effect of different grain sources on production of liquid spawn and fruiting body of *Cordyceps militaris*. *Intl J Agric Biol* 28:67–74

fermentation due to *Cordyceps militaris* has higher cordycepin content compared to other species of the genus *Cordyceps* (Raethong *et al*. 2020). In another study, Amin *et al*. (2008) reported that the mycelium of mushrooms cultivated in various agro-wastes could be infected, whereas that mycelium produced in the culture medium might be free from contamination. Mycelial development is stimulated by a growth medium, which provides quality and year-round productivity. There has been little information available on the effect of various parameters of temperature, humidity and incubation time on the mycelial production of *Cordyceps* and cordycepin using various solid substrates i.e. fermentation of rice, wheat and oat. There are primarily two types of modeling techniques for cultivating fruit bodies: those that use insects as hosts and those that use cereal grains (mostly rice or wheat) as substrates. The latter is more popular due to its ease of use and fruiting body products formed with this model compete more effectively. China produces around 4000 tones of dry fruit bodies every year, utilizing at least 500,000 tones of grain substrates (Lin *et al*. 2017). Artificial media have been synthesized to extract bioactive compounds for mycelial biomass and fruiting body production. According to Hong *et al*. (2010), the natural host insects for artificial cultivations are costly, difficult to manage, unavailable and susceptible to microbial pathogens and are threatened with extinction in nature.

In Pakistan, there is no any research work conducted on the production of this medicinal mushroom therefore, this research aimed to study the effect of liquid spawn on different soaked and unsoaked substrates of wheat, rice and sorghum and also optimized the growth of mycelium and production of the fruiting body of *C. militaris* under different growing conditions.

Materials and Methods

Collection of the fruiting body

Dry fruiting body of *C. militaris* used in the present study was obtained from Pakistan Museum of National History Islamabad, washed with 5% Sodium hypochlorite solution three times and dried on sterilized blotter paper and incubated on PDA supplemented with 0.5 g $MgSO_4$ for 10 days at 25 ± 1°C in the darkness for mycelial growth (Kang *et al*. 2012).

Preparation of liquid spawn from potato dextrose medium (PD)

Two hundred grams of potato were placed into 1000 mL water, boil 25 min, cross leached with muslin cloth afterward added glucose (30 g), peptone (5 g), yeast extract (3 g), KH_2PO_4 (1 g) and $MgSO_4.H_2O$ (0.5 g). The material was then autoclaved at 121°C for 15 min, cooled down subsequently from the PDA plate, the regions of the mycelial tip of *C. militaris* were punched out approximately 6 mm of PDA discs and transferred into PD. A total of four treatments were set for PD for the growth of liquid spawn. The details of the treatments were as:

PD 1 = PD + Streptomycin (5 mL L^{-1} in PD) + vitamin B_6 and B_{12} (0.5 mL L^{-1} in PD)
PD 2= PD + Streptomycin (5 mL L^{-1} in PD)
PD 3=PD + vitamin B_6 and B_{12} (0.5 mL L^{-1} in PD)
PD 4= PD without any treatment (Control)

This mixture was cultured on a rotary shaker incubator (Daihan Scientific, Korea) under conditions of 25 ± 1°C and darkness with shaking at 150 rpm min^{-1} for 10 days and measured the diameter (cm) of mycelium.

Determination of mycelial weight

Filtration of liquid cultures was done with pre-weighed filter papers (What man, Germany) to separate mycelium from liquid culture of PD, then the mycelium was weighed (g).

Preparation of soak and unsoaked grains for mycelial growth

Grain sources consisting of brown rice, sorghum and wheat (1000 g each) were used during the study. All grains were rinsed three times in clean water to remove dust and other particles, soaked in water for four hours to ensure maximum water absorption. Soaked grains were washed again whereas unsoaked grains were washed without soaking. Both soaked and unsoaked grains were filled @ 30 g into three different treated spawn glass jars and supplemented with PD.

Preparation of PD with egg mixture basal medium for substrate

Two blended eggs and glucose (30 g), peptone (5 g), yeast extract (3 g), KH_2PO_4 (1 g), $MgSO_4.H_2O$ (0.5 g), vitamin B_6 and B_{12} (0.5 mg) were added in 1000 mL potato broth and mixed well with hand beater for ten minutes.

Container selection for optimization of fruiting body cultivation

Three types of treatments were selected for cultivation and optimization of the fruiting body in a glass container.
A = A glass container covered with filter paper.
B = A glass jar with a hole in the plastic lid (A hole was punched in the plastic lid about 1.5 cm in diameter and sealed with cotton).
C = A glass container covered with a polythene sheet.

Autoclaving of the supplemented glass jar

Soaked and unsoaked grains were filled into each glass jar with 20 g grain and 30 mL PD basal medium and sterilized in autoclave at 121°C for 15 min. After cooling, each glass

Table 1: Mycelial growth of *C. militaris* under different treatments in liquid spawn

Treatment		Diameter (cm)	Days Required for spawn completion	Thickness of mycelium (mm)	Fresh Biomass (g/200 mL) liquid culture
PD 1	PD + Streptomycin+ vitamin B_6 and B_{12}	4.5±0.03	11 ± 0.00	5.2 ± 0.02	10.2 ± 0.04
PD 2	PD +Streptomycin	4.1±0.05	15 ± 0.01	4.3 ± 0.03	8.3 ± 0.05
PD 3	PD+ vitamin B_6 and B_{12}	3.8±0.02	20 ± 0.01	4.1 ± 0.02	6.5 ± 0.03
PD 4	PD (Control)	3.5±0.03	25 ± 0.03	2.5 ± 0.03	5.1 ± 0.02

Table 2: Effect of different soaked and unsoaked substrates on the average growth and development of *C. militaris* at 23 ± 2°C

Substrate		Treatments	Mycelial growth time (days)	pinhead occurrence time (days)	fruiting body mature time (days)	length of fruiting (cm)
Soaked	Wheat	plastic cap (T1)	6	40	70	2 ± 0.03
		filter paper (T2)	7	0	0	0 ± 0.00
		polythene sheet (T3)	5	35	65	3 ± 0.03
	Sorghum	plastic cap (T4)	6	36	60	2.5 ± 0.05
		filter paper (T5)	6	0	0	0 ± 0.00
		polythene sheet (T6)	6	32	58	3 ± 0.08
	Brown rice	plastic cap (T7)	4	30	56	3.3 ± 0.08
		filter paper (T8)	5	0	0	0 ± 0.00
		polythene sheet (T9)	4	27	52	3.8 ± 0.05
Un soaked	Wheat	plastic cap (T10)	7	50	85	1.2 ± 0.03
		filter paper (T11)	9	0	0	0 ± 0.00
		polythene sheet (T12)	6	46	82	1.5 ± 0.05
	Sorghum	plastic cap (T13)	6	45	80	1.7 ± 0.08
		filter paper (T14)	7	0	0	0 ± 0.00
		polythene sheet (T15)	6	42	77	1.8 ± 0.03
	Brown rice	plastic cap (T16)	6	40	75	2 ± 0.03
		filter paper (T17)	6	0	0	0 ± 0.00
		polythene sheet (T18)	5	38	71	2.5 ± 0.05

jar was inoculated with 10 mL liquid spawn of *C. militaris*, incubated at 25 ± 1°C under dark conditions and replicated 5 times. The diameter of the mycelium growth was measured after every 5 days for 25 days. After a period of 12 days were kept out of the dark and placed in artificial light (400–500 lux) for about 12 h per day to initiate fruiting under temperature between 23 ± 2°C for 60 days. After fruiting fresh fruiting bodies were collected, weighted (mg) and measured (cm).

Statistical analysis

The data were statistically assessed using one-way analysis of variance (ANOVA) on version 20.0 software (SPSS Inc., Chicago, USA). All values were expressed as mean ± standard error (SE) as suggested by Wiengmoon *et al*. (2019).

Results

Effect of different treatments of liquid spawn on mycelial growth of *C. militaris*

The maximum, thick and the highest diameter (4.5 ± 0.03 cm) of mycelial growth in liquid spawn was observed in PD containing antibiotic streptomycin with vitamins (PD1) at 25 ±1°C (RH, 70–75) with the highest biomass (10.2 ± 0.04 g) within 11 days whereas, the minimum (5.1 ± 0.02) was noticed under PD4 treatment. The order followed as PD1 < PD2 < PD3 < PD4 (Table 1). The fungal mycelium of *C. militaris* was white, dense and more or less completely covered the surface of the culture broth (Fig. 1).

Effect of soaked and unsoaked grain sources on mycelium growth of *C. militaris*

C. militaris showed varied mycelial growth on the three different kinds of grains. In terms of mycelial growth, it took 4 to 9 days to cover the whole soaked and unsoaked substrates of rice, wheat and sorghum. The result showed that the earliest mycelial growth was recorded for soaked brown rice for the treatment (T7) and T9. Initial pinhead development was noticed for T9 and late for T1 on rice and wheat substrate respectively. In comparison with rice media, pinheads took a longer time to mature for wheat and sorghum substrate. However, the lengths of fruit bodies on rice media were recorded greater than those on the other groups. The highest length of fruiting bodies was recorded for T9 and the lowest under T10 whereas, no growth was observed for T2, T5, T8, T11, T14 and T17 (Table 2). Among the three kinds of soaked and unsoaked substrates, the highest yield of 16.3 g and 13.3 g from soaked rice substrate was recorded in T9 and T7 respectively followed by the second-highest yield of 13 g (T6) under sorghum whereas, the lowest yields (8 g) were recorded for wheat (T3). In the case of unsoaked substrate T18, T13 and T12 yielded the highest fruiting body for brown rice, sorghum and wheat respectively. The highest fresh weight (16.3 g) of *C. militaris* was recorded for soaked rice (T9) and the lowest (2.1 g) for unsoaked wheat grain (T10), (Fig. 2).

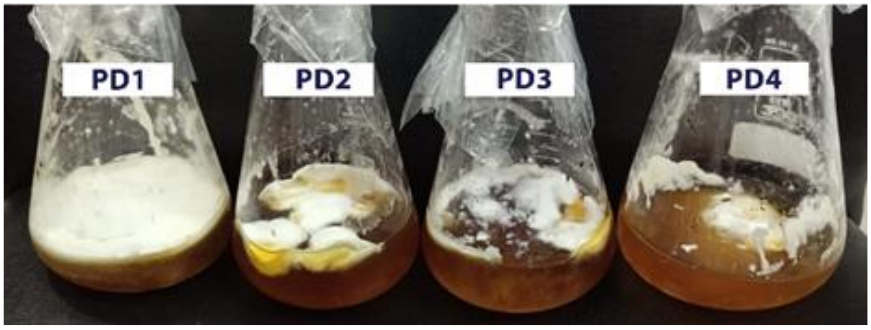

Fig. 1: Mycelial growth of *C. militaris* affected by different PD treatments in liquid spawn. (PD 1 = PD + Streptomycin + vitamin B6 and B12, PD 2= PD + Streptomycin, PD 3=PD + vitamin B6 and B12 PD 4= Control)

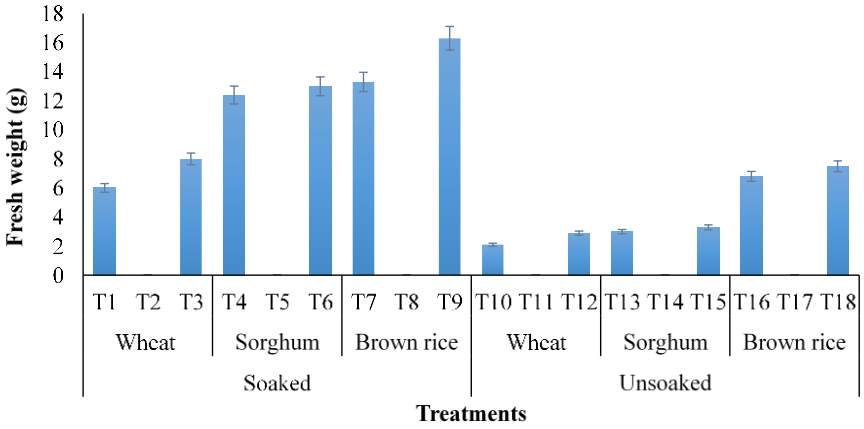

Fig. 2: Effect of different soaked and unsoaked substrates on the yield of *C. militaris*
(T1,T4,T7,T10,T13,T16(plastic cap),T2,T5,T8,T11,T14,T17(Filter paper),T3,T6,T9,T12,T15,T18(polythene sheet)

Effect of different glass jars on the fruit body production of *C. militaris*

The result showed that amongst all treatments of glass jar the fastest mycelial growth, early pin head development, fruiting body maturity time, length and weight of fruiting bodies of *C. militaris* were recorded in glass jars sealed with polythene sheet for soaked and unsoaked substrates of rice, sorghum and wheat followed by glass jars capped with plastic lids. However, no growth of *C. militaris* was noticed in a glass jar covered with filter paper (Fig. 3).

Discussion

The present study was conducted with the purpose to assess the suitable, profitable and inexpensive growth conditions for the cultivation of *C. militaris*. The results suggested that mycelial development of *C. militaris* is influenced by cultural conditions and available nutrients. Mycelial growth diameter on PDA medium supplemented with $MgSO_4$ was tested in the current study, and it was discovered that it not only increased mycelial growth diameter but also reduced the cultivation period. Our findings were consistent with those of Maftoun *et al*. (2013), who found that Potato dextrose agar medium (PDA) is the optimal medium for high fungal mycelial development and is extensively used as a standard for spore inoculum formation of mushrooms. Our results were in accordance with Shrestha *et al*. (2006) who tested twenty-two different types of media and classified them as deficient or rich in nutritional elements and found that PDA and C-DOX exhibited high fungal intensity. Similarly, Wongsorn *et al*. (2021) observed that nutritionally rich PDA medium resulted in abundant mycelium development of *C. militaris*. The present study suggested that the mycelial growth of *C. militaris* was found best in PDA medium at 25 ± 1°C. The findings of the present study were found to be comparable with Adnan *et al*. (2017) who studied that the mycelium progression of *Cordyceps* was greatest in PDA media at 20ºC and 25ºC.

During the study, the maximum, thick and highest diameter of mycelial biomass in liquid spawn was observed in PD containing antibiotic streptomycin with vitamins B_6 and B_{12} in minimum days at 25 ± 1°C (RH, 70–75). In another study, vitamins B_1, B_6, B_8, B_9 and B_{12} were reported to give the significantly highest mycelium colony diameter for the production of *C. militaris* (Wen *et al*. 2011; Ma *et al*. 2015; Dang *et al*. 2018). Our observations also matched those of Rozsa and Apahidean (2020), who reported that the optimal incubation temperature for obtaining the highest mycelial biomass was in the 24–28°C

Table 3: F-ratio and significant levels derived from ANOVA for all treatments

Factors	SS	d.f.	MS	F-value	Significance
Substrates	105.000	12	8.750	6.833	***0.001
	52.500	41	1.280		
	157.500	53			
Treatments	8829.000	12	735.750	7.094	***0.001
	4252.500	41	103.720		
	13081.500	53			
Biomass of *C. militaris*	494.973	5	98.995	4.681	***0.001
	1015.120	48	21.148		
	1510.093	53			

F = F – ratio, P = P-value (****P* ≤ *0.001*, ** *P* ≤ *0.01*, *P* ≤ *0.05*, ns=non-significant)

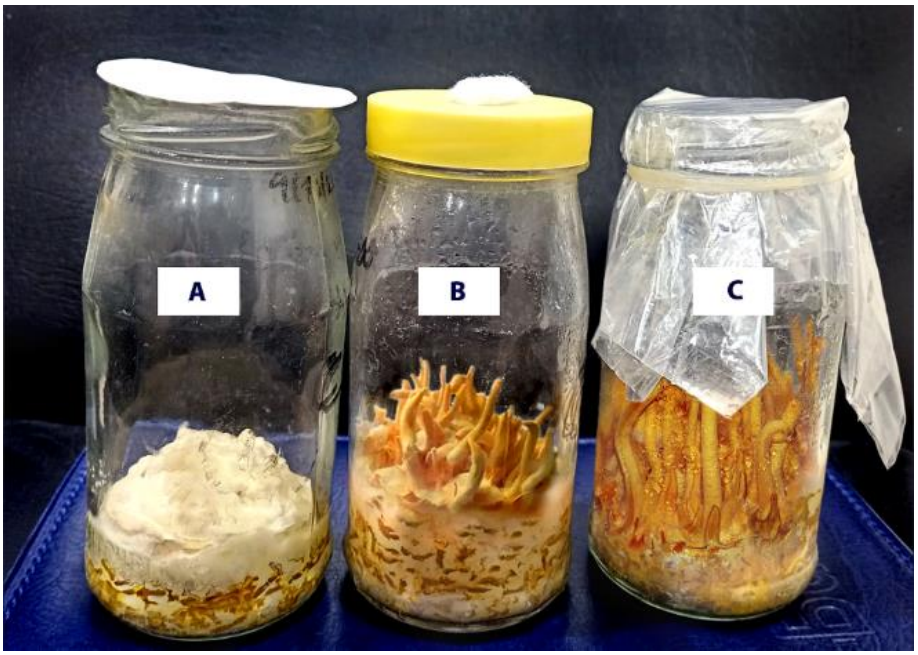

Fig. 3: Fruiting body production of *Cordyceps militaris* in different glass jars
(A= glass jar covered with filter paper, B= glass jar with a hole in the plastic lid, C= covered with polythene sheet.)

temperature range. Sasaki *et al*. (2005) also reported that the mycelial growth of *C. sinensis* was better at 20ºC and 25ºC whereas no growth was recorded at 30ºC and 35ºC. In the present research, liquid spawn was developed in a shaking flask, which is a low-cost approach using basic equipment technology and is appropriate for the mass production of fungal biomass. According to Lee *et al*. (2013), mycelial biomass powder can be utilized to make several types of supplementary capsules and tablets.

In this study, various grains like brown rice, wheat and sorghum were included to evaluate their effects on the mycelium production of *C. militaris*. The results indicated that mycelium extensions of wheat and sorghum were significantly slower than brown rice treatments. Mycelium densities of brown rice on grain media were compact and somewhat compact for both wheat and sorghum. Brown rice was found to be the most favorable to the mycelium growth and production of the fruiting body of *C. militaris*. The highest and thick mycelium was obtained from brown rice and next to sorghum and wheat. This is probably due to the relatively higher content of starch in brown rice and addition with eggs mixture improved mycelial fruiting production. Furthermore, brown rice can retain water to support the growth and supplement the mycelial growth of *C. militaris*. Many researchers cultivated *C. militaris* using different varieties of rice including black jasmine rice, white rice, brown rice, sao hai rice, black glutinous rice, Thai jasmine rice, and sangyod brown rice (Tianzhu *et al*. 2015; Sornprasert *et al*. 2016). In another study, Adnan *et al*. (2017) investigated the most efficient method for boosting cordycepin production on a wide scale and discovered that rice medium had the greatest cordycepin production (814.60 mg g^{-1}) followed by oat (638.85 mg g^{-1}) and wheat (565.20 mg g^{-1}) medium respectively. The mycelium colony diameter of *C. militaris* in different grains was significantly affected by substrate type while larger surface area and spore of substrates were responsible for enhanced mycelium growth rate (Sofi *et al*. 2014). According to Qin and Han (2013), the

Cordyceps can be grown in many types of solid culture medium, the cultivation rate and volume of the hyphae were greatest in a solid culture medium of rice as the solid substrate in contrast with other solid media. The artificial growth of *C. militaris* was effectively conducted by Pathania and Sagar (2014) under lab-scale cultivation trials on wheat and maize grains substrates. Wu *et al*. (2022) examine the impact of seven-grain substrates on the development of C. *militaris* fruiting bodies using solid-state fermentation and observed that brown rice and buckwheat had greater levels of adenosine and pentostatin.

The present experiment was performed by culturing the *C. militaris* on three different substrates with three modifications of glass jars covered with filter papers, plastic sheets and plastic lids. The results showed that amongst all treatments the highest mycelial growth rate and fresh yield of *C. militaris* were recorded in glass jars concealed with a polythene sheet for soaked and unsoaked substrates of rice, sorghum and wheat. This happened due to the favorable effect of temperature, light and humidity on glass jars covered with a transparent plastic sheet as compared to others i.e. plastic lids and filter papers. This indicated that the modified plastic sheet jars could be beneficially used as culturing jars for *Cordyceps* cultivation and could be a substitute for the expensive commercial growth jars. Our results were also in conformity with Wiengmoon *et al*. (2019) who confirmed that the growth jar is the best suitable application to yield high quality and quantity of fruiting body of *C. militaris* than the culture room because it is cheap and easily manageable. The results further showed that the highest mycelium biomass and fruiting body production period was found best on 23 ± 2°C (RH 80–90%) in all tested substrates. Several authors mentioned the more or less temperature of 24°C as optimal for incubating and growing the mycelium of the *C. militaris* mushroom (Mao *et al*. 2005; Patel and Ingalhalli 2013), while, Guo *et al*. (1998) and Masuda *et al*. (2007) stated optimal temperature of 28°C. According to Mani *et al*. (2015), the mycelial biomass and fruiting production were recorded optimum at 20°C, yielding 10.5 ± 0.14 and 1.75 ± 0.99 g L^{-1}. The temperature range for fruiting body cultivation of *C. militaris* was found between 18°C to 25°C. The optimal temperature of 20°C and 25°C was also described by (Cheng *et al*. 2011). Yoo *et al*. (2022) developed a technique for artificially cultivating *C. militaris* using germinated soybeans rather than pupae as a protein source at 25°C.

The present study found that brown rice was found best substrate for the mycelium growth and production of *C. militaris* in soaked and unsoaked treatments in glass jars wrapped with a polythene sheet. The analysis of variance demonstrated that the mycelial growth of *C. militaris* in different treatments at 0.01 level showed a significant difference and its effect on different soaked and unsoaked substrates and fruiting body production were found highly significantly different for all levels ($P \leq 0.001$) (Table 3). Based on the production and quality of fruiting bodies, brown rice substrate remained suitable for the cultivation of *C. militaris* similar to those observed on rice substrate, the other next suitable substitute substrate was sorghum, which supported the moderate production of fruit bodies, however, wheat proved the lowermost in the cultivation of *C. militaris*.

Conclusion

It is concluded from the results obtained that the mycelial and fruiting growth rates of *C. militaris* were significantly highest and fastest on the rice substrate incubated in the polythene glass jars followed by jars covered with plastic lids and filter papers. Further, using a polythene sheet could reduce production expenses and help protect the environment. However, further research in Pakistan is necessary to optimize cultivation factors such as light, incubation time, aeration and temperature.

Acknowledgments

The author is grateful to the laboratory attendant of Food quality and safety research institute Mr. Zafar Hamid Khan and Mr. Israr Ahmed Ansari (Scientific Assistant) for their technical support during the study period.

Author Contributions

US and PAB planned and performed the experiments, US and MIB interpreted and reviewed the results, US and AK statistically analyzed the data and revised the result

Conflicts of Interest

The authors declare no conflict of interest.

Ethics Approval

Not applicable in this paper

References

Adnan M, SA Ashraf, S Khan, E Alshammari, AM Awadelkareem (2017). Effect of pH, temperature and incubation time on cordycepin production from *Cordyceps militaris* using solid-state fermentation on various substrates. *CyTA J food* 15:617–621

Amin SR, N Alam, M Tania, MA Khan (2008). Study of mycelial growth of *Cordyceps sinensis* in different media, at different PH level and temperature. *Bang J Mushr* 2:43–48

Bajwa R, R Kausar, A Javaid (1999). Yield performance of *Pleurotus ostreatus* (oyster mushroom) cultivated on cereal crop residues amended with *Sesbania sesban* leaves. *In: Proceedings of 2nd National Conference of Plant Pathology*, pp:160–164. September 27–29, 1999. University of Agriculture Faisalabad, Pakistan

Berovic M, BB Podgornik, A Gregori (2022). Cultivation technologies for production of medicinal mushroom biomass. *Intl J Med Mushr* 24:1–22

Chen YS, BL Liu, YN Chang (2011). Effects of light and heavy metals on *Cordyceps militaris* fruit body growth in rice grain-based cultivation. *Kor J Chem Eng* 28:875–879

Cheng H, W Guo, M Chang, J Meng, J Yang (2011). Study of optimization on liquid fermentation conditions of *Cordyceps militaris* mycelium. *J Shan Agric Univ* 31:66–72

Dang HN, CL Wang, HL Lay (2018). Effect of nutrition, vitamin, grains, and temperature on the mycelium growth and antioxidant capacity of *Cordyceps militaris* (strains AG-1 and PSJ-1). *J Rad Res Appl Sci* 11:130–138

Das G, HS Shin, G Leyva-Gómez, MLD Prado-Audelo, H Cortes, YD Singh, MK Panda, AP Mishra, M Nigam, S Saklani, PK Chaturi, M Martorell, N Cruz-Martins, V Sharma, N Garg, R Sharma, JK Patra (2021). *Cordyceps* spp.: A review on its immune-stimulatory and other biological potentials. *Front Pharmacol* 11:1–31

Guo C, J Zhu, C Zhang, L Zhang (1998). Determination of adenosine and 3'-deoxyadenosine in *Cordyceps militaris* (L.) Link. by HPLC. *Chin J Chin Mater Med* 23:236–237

Hong IP, PD Kang, KY Kim, SH Nam, MY Lee, YS Choi, NS Kim, HK Kim, KG Lee, RA Humber (2010). Fruit body formation on silkworm by *Cordyceps militaris*. *Mycobiology* 38:128–132

Kang C, TC Wen, JC Kang, YX Qian, BX Lei (2012). Effects of additives and different culture conditions on cordycepin production by the medicinal fungus *Cordyceps militaris*. *Mycosystema* 31:389–397

Kontogiannatos D, G Koutrotsios, S Xekalaki, GI Zervakis (2021). Biomass and cordycepin production by the medicinal mushroom *Cordyceps militaris* – a review of various aspects and recent trends towards the exploitation of a valuable fungus. *J Fungi* 7:986–1003

Lee BJ, M Lee, YG Kim, KW Lee, YS Choi, BE Lee, HY Song (2013). Cultural characteristics of *Cordyceps militaris* strain'Yedang 3'on various media and nutritional conditions. *J Mushr* 11:124–130

Li X, J Wang, H Zhang, L Xiao, Z Lei, SC Kaul, Z Zhang (2021). Low dose of fluoride in the culture medium of *Cordyceps militaris* promotes its growth and enhances bioactivities with antioxidant and anticancer properties. *J Fungi* 7:1–14

Lin Q, L Long, L Wu, F Zhang, S Wu, W Zhang, X Sun (2017). Evaluation of different agricultural wastes for the production of fruiting bodies and bioactive compounds by medicinal mushroom *Cordyceps militaris*. *J Sci Food Agric* 97:3476–3480

Ma L, S Zhang, M Du (2015). Cordycepin from *Cordyceps militaris* prevents hyperglycemia in alloxan-induced diabetic mice. *Nutr Res* 35:431–439

Maftoun P, R Malek, M Abdel-Sadek, R Aziz, HE Enshasy (2013). Bioprocess for semi-industrial production of immunomodulator polysaccharide Pleuran by *Pleurotus ostreatus* in submerged culture. *J Sci Industr Res* 72:655–662

Mani A, J Patel, S Kalam, R Singh, SS Sandhu (2015). Evaluation of mycelial and exopolysaccharide production from *Cordyceps militaris*. *Inter J Appl Sci Eng Res* 4:609–619

Mao XB, T Eksriwong, S Chauvatcharin, JJ Zhong (2005). Optimization of carbon source and C:N ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. *Proc Biochem* 40:1667–1672

Masuda M, E Urabe, H Honda, A Sakurai, M Sakakibara (2007). Enhanced production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. *Enz Microb Technol* 40:1199–1205

Mehra A, KU Zaidi, A Mani, V Thawani (2017). The health benefits of *Cordyceps militaris*-A review. *Kavaka* 48:27–32

Panda AK, KC Swain (2011). Traditional uses and medicinal potential of *Cordyceps* sinensis of Sikkim. *J Ayurv Integr Med* 2:9–13

Patel KJ, RS Ingalhalli (2013). *Cordyceps militaris* (L.Fr.) Link – An Important Medicinal Mushroom. *J Pharmacol Phytochem* 8192:315–319

Pathania P, A Sagar (2014). Studies on the biology of *Cordyceps militaris*: A medicinal mushroom from northwest Himalaya. *Kavaka* 43:35–40

Qin X L, DM Han (2013). The Optimization Study on Solid Culture Medium of North *Cordyceps*. *Adv Mater Res* 781:884–888

Raethong N, H Wang, J Nielsen, W Vongsangnak (2020). Optimizing cultivation of *Cordyceps militaris* for fast growth and cordycepin overproduction using rational design of synthetic media. *Comput Struct Biotechnol J* 18:1–8

Raethong N, K Laoteng, W Vongsangnak (2018). Uncovering global metabolic response to cordycepin production in *Cordyceps militaris* through transcriptome and genome-scale network-driven analysis. *Sci Rep* 8:1–13

Rozsa M, M Apahidean (2020). Influence of temperature and PH level on mycelial growth in liquid cultures of *Cordyceps militaris* mushroom mycelium. *Curr Trend Nat Sci* 9:42–46

Rozsa S, DN Măniuțiu, G Poșta, TM Gocan, I Andreica, I Bogdan, M Rozsa, V Lazăr (2019). Influence of the culture substrate on the *Agaricus blazei Murrill* mushrooms vitamins content. *Plants* 8:316–334

Rozsa S, DM Măniuțiu, TM Gocan, R Sima, I Andreica, M Rózsa (2017). Mycelial biomass production of the Sun mushroom (*Agaricus blazei* Murrill). *Curr Trend Nat Sci* 6:126–130

Sasaki F, T Miyamoto, Y Tamai, T Yajima (2005). Optimum Temperature and pH for Mycelial Growth of *Cordyceps* nutans Pat. (Ascomycetes). *Intl J Med Mushr* 7:301–304

Schwenzer H, E De Zan, M Elshani, R Van Stiphout, M Kudsy, J Morris, SP Blagden (2021). The novel nucleoside analogue ProTide NUC-7738 overcomes cancer resistance mechanisms *in vitro* and in a first-in-human phase I clinical trial. *Clin Canc Res* 27:6500–6513

Shi K, G Yang, L. He, B Yang, Q Li, S Yi (2020). Purification, characterization, antioxidant, and antitumor activity of polysaccharides isolated from silkworm *Cordyceps*. *J Food Biochem* 44:e13482

Shrestha B, SK Han, JM Sung, GH Sung (2012). Fruiting body formation of *Cordyceps militaris* from multi-ascospore isolates and their single ascospore progeny strains. *Mycobiology* 40:100–106

Shrestha B, WH Lee, SK Han, JM Sung (2006). Observations on some of the mycelium growth and pigmentation characteristics of *Cordyceps militaris* isolates. *Mycobiology* 34:83–91

Sofi B, M Ahmad, M Khan (2014). Effect of different grains and alternate substrates on oyster mushroom (*Pleurotus ostreatus*) production. *Afr J Microbiol Res* 8:1475–1479

Sornprasert R, S Aroonsrimorakot, Hambananda (2016). A. Cultivation of *Cordyceps militaris* using different cereal grains and local insects and inhibitory efficiency against *Trichophyton rubrum* and *Staphylococcus aureus*. *J Kmutnb* 26:239–251

Tatani K, M Hiratochi, N Kikuchi, Y Kuramochi, S Watanabe, Y Yamauchi, F Itoh, M Isaji, S Shuto (2016). Identification of adenine and benzimidazole nucleosides as potent human concentrative nucleoside transporter 2 inhibitors: Potential treatment for hyperuricemia and gout. *J Med Chem* 59:3719–3731

Tianzhu Z, Y Shihai, D Juan (2015). The effects of cordycepin on ovalbumin-induced allergic inflammation by strengthening Treg response and suppressing Th17 responses in ovalbumin-sensitized mice. *Inflammation* 38:1036–1043

Wen TC, GR Li, JC Kang, C Kang, KD Hyde (2014). Optimization of solid-state fermentation for fruiting body growth and cordycepin production by *Cordyceps militaris*. *Chiang Mai J Sci* 41:858–872

Wen TC, JC Kang, ZQ Liang, BX Lei (2011). Optimization of submerged culture conditions for mycelial growth and cordycepin production of medicinal fungus *Cordyceps militaris*. *Guizhou Sci* 31:1–12

Wiengmoon B, K Sujipuli, S Prasarnpun, S Chindaruksa (2019). Mycelial growth and fruiting body production of *Cordyceps militaris* in different culture chambers. *NU Intl J Sci* 16:58–68

Wongsorn D, T Surasilp, S Rattanasuk (2021). Effects of Edible Insects on the Mycelium Formation of *Cordyceps militaris*. *Pak J Biol Sci* 24:881–887

Wu CY, CH Liang, ZC Liang (2022). Enhanced production of fruiting bodies and bioactive compounds of *Cordyceps militaris* with grain substrates and cultivation patterns. *J Tai Inst Chem Eng* 132:104138

Yang S, L Jin, X Ren, J Lu, Q Meng (2014). Optimization of fermentation process of *Cordyceps militaris* and antitumor activities of polysaccharides *in vitro*. *J Food Drug Anal* 22:468–476

Yi ZL, WF Huang, Y Ren, E Onac, GF Zhou, S Peng (2014). LED lights increase bioactive substances at low energy costs in culturing fruiting bodies of *Cordyceps militaris. Sci Hortic Intl Soc* 175:139–143

Yoo CH, MA Sadat, W Kim, TS Park, DK Park, J Choi (2022). Comprehensive transcriptomic analysis of *Cordyceps militaris* cultivated on germinated soybeans. *Mycobiology* 50:1–11

Zhong X, L Gu, WT Xiong, HZ Wang, DH Lian, YM Zheng, S Zhou, W Zhou, JL Gu, JH Shen, J Wang, GR Zhang, X Liu (2020). 1H NMR spectroscopy-based metabolic profiling of Ophio *Cordyceps* sinensis and *Cordyceps militaris* in water-boiled and 50% ethanol-soaked extracts. *J Pharm Biomed Anal* 180:113038