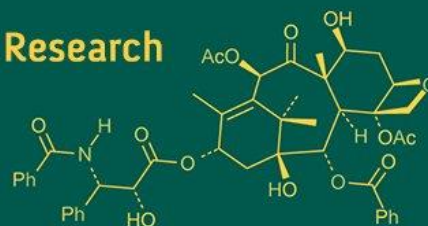
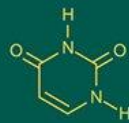


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(8): 2098-2105
www.biochemjournal.com
Received: 17-06-2026
Accepted: 19-07-2026

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Cost effective evaluation of liquid growth media for submerged spawn production of *Cordyceps militaris* (L.) Link

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i8Sw.9714>

Abstract

Cordyceps militaris (L.) Link is a commercially important medicinal fungus recognized for its diverse bioactive compounds and broad pharmaceutical and nutraceutical applications. This study evaluated different liquid culture media to identify a nutritionally efficient and economically feasible medium for submerged liquid spawn production of *C. militaris*. Nine liquid culture media were compared using a Completely Randomized Design with three replications. Mycelial growth was evaluated after 14 days of submerged cultivation by measuring pellet volume and optical density (OD₆₀₀), while the raw material cost of each medium was also estimated. Considerable variations were observed among the media tested. Jaggery Peptone broth (T₃) produced the highest pellet volume (4.750 ml), followed by Malt extract broth (2.667 ml) and Jaggery broth (2.500 ml). The highest optical density (2.628) was recorded in Jaggery dextrose peptone broth (T₃), which was statistically at par with Jaggery peptone broth (2.527) and the standard *Cordyceps* liquid medium (2.473). The raw material cost of the evaluated media ranged from ₹15.00 to ₹212.90 per litre. Although Jaggery peptone broth required only ₹51.50 per litre for preparation, compared with ₹77.00 per litre for the standard medium, it produced the greatest pellet volume among all treatments. These results suggest that a balanced nutrient composition is more important for submerged mycelial growth than the complexity or cost of the culture medium. Overall, Jaggery peptone broth proved to be a simple, effective and comparatively economical medium for laboratory scale submerged liquid spawn production of *C. militaris*.

Keywords: *Cordyceps militaris*, cost effective medium, liquid spawn, mycelial growth, optical density, pellet volume, submerged cultivation

Introduction

Cordyceps militaris (L.) Link is an entomopathogenic medicinal fungus belonging to the family Cordycipitaceae and is recognized as one of the most commercially important fungi used in functional foods, nutraceuticals and pharmaceutical research. Unlike the highly valued caterpillar fungus *Ophiocordyceps sinensis* whose commercial cultivation is limited by host specificity, ecological requirements and declining natural populations, *C. militaris* can be cultivated successfully under artificial conditions while producing many of the same pharmacologically important metabolites. Its ease of cultivation, shorter production cycle and adaptability to both solid-state and submerged culture systems have established it as a practical and sustainable alternative to *O. sinensis* (Shrestha *et al.*, 2012; Kontogiannatos *et al.*, 2021). The species is rich in biologically active compounds, including cordycepin, adenosine, polysaccharides, ergosterol, ergothioneine, carotenoids, peptides and essential amino acids. Among these, cordycepin has attracted particular attention because of its anticancer, antioxidant, antimicrobial, antiviral, anti-inflammatory, immunomodulatory and neuroprotective properties, making *C. militaris* an important industrial microorganism for the production of high value bioactive compounds (Chan *et al.*, 2015; Kontogiannatos *et al.*, 2021; Park, 2025) [1, 9, 13, 16].

Commercial cultivation of *C. militaris* is carried out through solid-state cultivation for fruiting body production and submerged liquid cultivation for mycelial biomass and liquid spawn production. Among these, submerged cultivation offers several advantages, including rapid

biomass production, uniform nutrient availability, improved oxygen transfer, better control of culture conditions and suitability for large-scale fermentation. More importantly, it enables the production of liquid spawn, which serves as a physiologically active inoculum for solid-state cultivation, facilitating rapid substrate colonization, uniform mycelial establishment and improved crop uniformity. Consequently, submerged liquid cultivation has become an integral component of modern *Cordyceps* cultivation systems, particularly for the preparation of high-quality liquid spawn used in commercial fruiting body production (Kim & Yun, 2005; Shih et al., 2007; Wen et al., 2012)^[7, 15, 19].

The success of submerged cultivation largely depends on the nutritional composition of the culture medium, which directly influences fungal metabolism, hyphal extension, pellet formation, biomass accumulation and ultimately the quality of the liquid spawn. Carbon and nitrogen sources provide the essential substrates for energy production, protein synthesis and cellular growth, whereas vitamins and mineral salts regulate various metabolic processes involved in fungal development. Pellet morphology is another important characteristic of submerged cultures, as pellet size and structure influence oxygen transfer, nutrient diffusion, mixing efficiency and inoculum handling. Since liquid spawn serves as the inoculum for solid-state cultivation, its physiological quality is critical for rapid substrate colonization and uniform mycelial establishment. Therefore, selecting an appropriate liquid culture medium is essential for producing healthy, vigorous and high-quality liquid spawn for subsequent fruiting body cultivation (Dong & Yao, 2005; Mao et al., 2006; Park et al., 2002; Shih et al., 2007)^[12].

Considerable research has focused on optimizing submerged cultivation of *Cordyceps* species by modifying carbon and nitrogen sources, carbon-to-nitrogen ratio, mineral nutrition, pH, inoculum concentration and fermentation conditions to enhance mycelial biomass and cordycepin production (Dong & Yao, 2005; Mao et al., 2005; Shih et al., 2007; Wen et al., 2012; Raethong et al., 2020)^[4, 10, 11, 14, 15, 19]. However, comparatively little attention has been paid to the development of nutritionally efficient and cost-effective liquid culture media for routine liquid spawn production. Most commonly used media rely on multiple laboratory-grade ingredients, increasing preparation costs and reducing their practicality for routine use. Furthermore, limited information is available on whether simpler nutrient formulations can produce high-quality liquid spawn capable of supporting rapid substrate colonization while maintaining desirable pellet formation and mycelial growth. Therefore, identifying an effective and comparatively economical liquid culture medium remains an important prerequisite for improving liquid spawn production for subsequent solid-state cultivation.

Therefore, the present investigation was undertaken to compare a standard liquid culture medium with eight alternative media differing in nutrient composition for the production of *Cordyceps militaris* liquid spawn under submerged cultivation. Pellet volume and optical density were used as indicators of mycelial growth and liquid spawn quality, while the raw material cost of each medium was estimated to compare preparation costs. The study aimed to identify a nutritionally effective and comparatively economical liquid culture medium capable of producing high-quality liquid spawn for efficient substrate colonization during subsequent solid-state cultivation and to provide a scientific basis for the further optimization of liquid culture protocols for laboratory and commercial applications.

Materials and Methods

Experimental site

The experiment was conducted in the Department of Plant Pathology, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani, Maharashtra, India. All experimental procedures were carried out under aseptic and controlled laboratory conditions.

Procurement of fungal culture and maintenance

Pure culture of *Cordyceps militaris* (L.) Link was procured from Sunrise Enterprises-Mushroom Learning Centre, Biobritte Agro Solutions Pvt. Ltd., Kolhapur, Maharashtra, India. The culture was maintained on Potato Dextrose Agar (PDA) at 18 °C and sub-cultured once in three months to preserve its purity and viability. Actively growing 10-day-old cultures were used as the inoculum source for all subsequent experiments (Fig. 1).

Preparation of liquid culture media

Nine liquid culture media differing in nutrient composition were evaluated for submerged liquid spawn production of *Cordyceps militaris* as mentioned in the Table 1. The standard *Cordyceps* liquid medium (T₁), routinely employed for submerged spawn production, served as the reference treatment, while eight additional media with different carbon and nitrogen based nutrient compositions were selected for comparative evaluation. The composition of each liquid culture medium is presented in Table 1. The required quantities of individual ingredients were suspended in distilled water. Thereafter, 250 ml of each medium was dispensed into sterile 500 ml Erlenmeyer flasks. The pH of the media was not adjusted before sterilization. The flasks were sterilized in an autoclave at 121°C under 15 psi for 15-20 min and allowed to cool to room temperature before inoculation. The formulation of the standard *Cordyceps* liquid medium and the submerged cultivation procedure were adopted from previously reported protocols for *C. militaris* cultivation (Mao et al., 2005; Wen et al., 2012)^[10, 19].

Cost-effectiveness of liquid culture media

The raw material cost of each liquid culture medium was estimated based on the quantity of individual ingredients required for preparing one litre of medium. Market prices of laboratory chemicals (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and locally procured food-grade ingredients were considered for cost estimation. Only the cost of media components was included, whereas expenses associated with distilled water, electricity, sterilization, labour, glassware and other consumables were excluded. The ingredient-wise cost and the total preparation cost of each medium are shown in Table 2.

Inoculation and incubation

Each flask was aseptically inoculated with a single 5 mm diameter mycelial agar disc excised from the actively growing margin of a 10 days old *C. militaris* culture using a sterile cork borer. All inoculations were carried out inside a laminar airflow chamber under aseptic conditions. The inoculated flasks were incubated at 18 °C on a rotary shaker operating at 140 rpm for 14 days under dark conditions to promote submerged mycelial growth and pellet formation.

Assessment of pellet volume

After 14 days of incubation, the submerged cultures were gently mixed to ensure uniform distribution of the mycelial biomass. From each replicate flask, a 10 ml aliquot of the

liquid culture was aseptically transferred into a sterile 10 ml graduated centrifuge tube and centrifuged at 5000 rpm for 5 min to separate the mycelial biomass from the culture broth. Following centrifugation, the supernatant was carefully decanted without disturbing the pellet. The volume of the compact mycelial pellet was measured directly from the graduations on the centrifuge tube and expressed in millilitres (ml). One observation was recorded from each of the three replicate flasks and the mean pellet volume was subjected to statistical analysis.

Assessment of optical density

Mycelial growth in the different liquid culture media was further assessed by measuring the optical density (OD) of the culture suspension using a UV-Visible spectrophotometer. The cultures were gently mixed before sampling and the absorbance of the culture from each replicate flask was recorded at 600 nm (OD₆₀₀). Optical density was used as an indirect estimate of fungal biomass, with higher absorbance values indicating greater mycelial growth under submerged culture conditions. One observation was recorded from each of the three replicate flasks and the mean values were subjected to statistical analysis.

Experimental design and statistical analysis

The experiment was conducted in a Completely Randomized Design (CRD) comprising nine liquid culture media as treatments with three replications. Data was recorded for pellet volume and optical density were subjected to analysis of variance (ANOVA), and treatment means were compared at the 5% level of significance according to Gomez and Gomez (1984). The standard error of the mean [SE(m)], standard error of the difference [SE(d)] and critical difference (CD) were calculated to evaluate treatment effects [6].

Results

Effect of liquid culture media on pellet volume

The composition of the liquid culture media significantly influenced the pellet volume of *Cordyceps militaris* after 14 days of submerged cultivation (Table 3). Considerable variation in pellet development was observed among the evaluated media. Jaggery-Peptide broth (T₃) produced the highest pellet volume (4.750 ml) and was significantly superior to all other treatments. This was followed by Malt Extract Broth (T₈) and Jaggery broth (T₂), which recorded pellet volumes of 2.667 and 2.500 ml, respectively, and were statistically at par with each other. Yeast Malt Extract Broth (T₇) exhibited an intermediate pellet volume (1.333 ml), whereas Jaggery-Dextrose-Peptide-Yolk broth (T₅) and the standard Cordyceps liquid medium (T₁) recorded comparatively lower pellet volumes of 1.000 and 0.917 ml, respectively. The lowest pellet volume (0.250 ml) was observed in Peptide-Yeast Extract Broth (T₉), followed by Jaggery-Yolk broth (T₄) (0.300 ml) and Sabouraud Dextrose Broth (T₆) (0.500 ml). As illustrated in the Fig. 2, Jaggery-Peptide broth produced the highest pellet volume despite requiring a comparatively lower preparation cost than several of the other evaluated media. The differences among treatments were statistically significant, as indicated by a critical difference (CD_{0.05}) of 0.286 ml.

Effect of liquid culture media on the optical density

The optical density (OD₆₀₀) of *Cordyceps militaris* differed significantly among the evaluated liquid culture media after 14 days of submerged cultivation (Table 4). The highest optical density (2.628) was recorded in Jaggery-Dextrose-

Peptide-Yolk broth (T₅) and was statistically at par with Jaggery-Peptide broth (T₃) (2.527) and the standard Cordyceps liquid medium (T₁) (2.473). Malt Extract Broth (T₈) also supported relatively high mycelial growth, recording an OD₆₀₀ value of 2.378, followed by T₇ i.e. Yeast Malt Extract Broth (2.111). Comparatively lower optical density values were observed in Peptide-Yeast Extract Broth (T₉) (1.804), Sabouraud Dextrose Broth (T₆) (1.655) and Jaggery broth (T₂) (1.610), while the lowest OD₆₀₀ (1.497) was recorded in Jaggery-Yolk broth (T₄). As illustrated in the Fig. 3, the observed variation in optical density among the evaluated liquid culture media indicates differences in mycelial biomass production under submerged cultivation. Statistical analysis confirmed significant differences among the treatments, with a critical difference (CD_{0.05}) of 0.150.

Comparative preparation cost of different liquid culture media:

The preparation cost of the evaluated liquid culture media varied considerably depending on the composition and quantity of the ingredients used (Table 2). The total raw material cost ranged from ₹15.00 per litre for Jaggery broth (T₂) to ₹212.90 per litre for Peptide-Yeast Extract Broth (T₉), representing approximately a 14-fold difference among the evaluated formulations. The standard Cordyceps liquid medium (T₁) required a preparation cost of ₹77.00 per litre, whereas the remaining media ranged from ₹15.00 to ₹212.90 per litre. Among the alternative formulations, Jaggery-Yolk broth (T₄) and Jaggery-Peptide broth (T₃) required comparatively lower preparation costs of ₹19.50 per litre and ₹51.50 per litre, respectively, while Yeast Malt Extract Broth (T₇), Jaggery-Dextrose-Peptide-Yolk broth (T₅), Malt Extract Broth (T₈), and Sabouraud Dextrose Broth (T₆) required ₹83.90 per litre, ₹105.40 per litre, ₹154.60 per litre, and ₹196.80 per litre, respectively. The highest preparation cost (₹212.90 per litre) was recorded for Peptide-Yeast Extract Broth (T₉), whereas Jaggery broth (T₂) was the least expensive medium evaluated. As illustrated in Fig. 2, substantial variation in preparation cost was observed among the evaluated liquid culture media, with Jaggery-Peptide broth providing the most favourable balance between preparation cost and pellet volume. Overall, the evaluated liquid culture media exhibited substantial variation in raw material cost, reflecting differences in the number and type of ingredients used for medium preparation.

Discussion

The present study demonstrated that the nutritional composition of liquid culture media markedly influenced the quality of *Cordyceps militaris* liquid spawn produced under submerged cultivation, resulting in significant differences in pellet volume and optical density among the evaluated media. The composition of the culture medium is widely recognized as a key factor governing fungal physiology because carbon and nitrogen sources provide the essential substrates for energy production, protein synthesis, enzyme activity and cell wall formation. The interaction between these nutrients determines hyphal extension, branching pattern, pellet architecture and overall mycelial development, all of which contribute to the physiological quality of liquid spawn. Since liquid spawn serves as the inoculum for subsequent solid-state cultivation, its quality is expected to influence substrate colonization, uniform mycelial establishment and overall cultivation performance. Similar findings have been reported in Cordyceps and other medicinal fungi, where optimization of medium composition significantly improved mycelial growth, fermentation efficiency and physiological

performance (Kim et al., 2003; Kim & Yun, 2005; Fang et al., 2013). Recent reviews have likewise highlighted medium optimization as one of the most effective approaches for improving submerged cultivation of *C. militaris* (Wang et al., 2022; Park et al., 2025) [5, 8, 13, 18].

Among the evaluated formulations, Jaggery-Peptide broth produced the highest pellet volume, indicating that an appropriate balance of readily available carbon and organic nitrogen favoured the development of compact and well-defined mycelial pellets. Jaggery provides sucrose together with naturally occurring minerals and micronutrients, whereas peptide supplies peptides, amino acids and soluble nitrogen that support protein synthesis and active hyphal growth. The combined availability of these nutrients likely promoted coordinated hyphal extension and aggregation, resulting in the formation of larger and more compact pellets. Such pellet morphology is desirable for liquid spawn production because compact and healthy pellets are expected to facilitate uniform inoculation and rapid substrate colonization during subsequent solid-state cultivation. In contrast, jaggery alone supported only moderate pellet formation, indicating that carbon availability without an adequate supply of organic nitrogen was insufficient to produce vigorous liquid spawn. Similar responses have been reported in submerged cultures of *C. militaris*, where balanced carbon-to-nitrogen nutrition enhanced fungal growth more effectively than simply increasing nutrient concentration (Fang et al., 2013; Deshmukh & Bhaskaran, 2024) [3, 5]. Likewise, studies on filamentous fungi have consistently shown that organic nitrogen sources, such as peptide and yeast-derived compounds, promote vigorous mycelial growth by supporting rapid protein synthesis and metabolic activity (Chang et al., 2024; Wang et al., 2022) [2-18].

An interesting observation of the present investigation was that the medium producing the largest mycelial pellets was not the same as the one exhibiting the highest optical density. Although Jaggery-Peptide broth recorded the maximum pellet volume, Jaggery-Dextrose-Peptide-Yolk broth produced the highest OD₆₀₀. This finding indicates that pellet volume and optical density represent different but complementary indicators of liquid spawn quality. Pellet volume primarily reflects compact hyphal aggregation and pellet development, whereas optical density is influenced by the amount of suspended and dispersed mycelial biomass present in the culture broth. Nutrient-rich media may therefore stimulate greater hyphal branching and fragmentation, resulting in increased broth turbidity without necessarily producing larger pellets. Similar relationships among nutrient composition, pellet morphology and biomass distribution have been described in submerged fermentation of filamentous fungi, where pellet size and structural

organization influence oxygen transfer, nutrient diffusion and overall fermentation performance (Veiter et al., 2018) [17]. Recent molecular studies in *C. militaris* have further demonstrated that pellet formation involves coordinated regulation of cell wall remodelling and stress-response pathways, suggesting that pellet morphology represents an adaptive physiological response rather than simply a consequence of increased biomass (Wu et al., 2026) [20]. Therefore, simultaneous assessment of pellet volume and optical density provides a more comprehensive evaluation of liquid spawn quality than either parameter alone, particularly when the inoculum is intended for subsequent solid-state cultivation.

The present study also revealed considerable variation in the composition and preparation cost of the evaluated liquid culture media. The standard *Cordyceps* liquid medium consisted of several laboratory-grade ingredients, including potato extract, dextrose, peptone, ammonium citrate, mineral salts and vitamin B₁, whereas Jaggery-Peptide broth contained only two principal components, jaggery and peptide. Despite its comparatively simple formulation, Jaggery-Peptide broth produced the highest pellet volume among all the media evaluated while requiring a preparation cost of ₹51.50 per litre, approximately 33% lower than the standard medium (₹77.00 per litre). These findings indicate that the production of high-quality liquid spawn depends more on an appropriate balance of nutrients than on the complexity or cost of the culture medium. Furthermore, the simpler formulation minimizes the requirement for multiple laboratory-grade chemicals, mineral salts and vitamin supplements, thereby simplifying medium preparation and reducing dependence on specialized ingredients for routine laboratory use. In contrast, Peptide-Yeast Extract Broth, which required the highest preparation cost (₹212.90 per litre), produced the lowest pellet volume, demonstrating that greater expenditure on medium preparation does not necessarily result in superior liquid spawn quality. Although the present investigation did not evaluate subsequent substrate colonization or commercial production performance, the results indicate that carefully selected alternative formulations can produce high-quality liquid spawn while reducing preparation costs and simplifying medium composition. Such formulations may therefore provide practical alternatives for routine laboratory-scale liquid spawn production and serve as a foundation for further optimization of submerged culture protocols. Nevertheless, additional investigations on dry biomass production, liquid spawn performance during substrate colonization, fruiting body yield, cordycepin biosynthesis and comprehensive techno-economic evaluation are required before these formulations can be recommended for commercial-scale cultivation (Park et al., 2025; Wang et al., 2022) [13, 18].

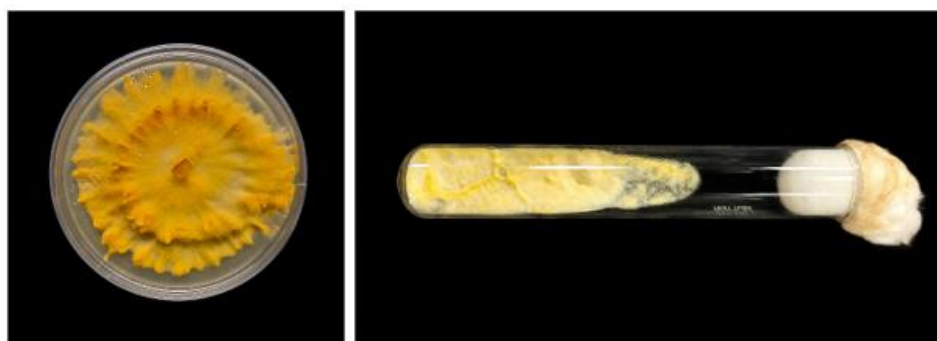


Fig 1: *Cordyceps militaris* (A) 10 days old mother culture (B) preserved PDA slant cultures

Table 1: Composition of liquid culture media for submerged liquid spawn production of *Cordyceps militaris*.

Treatment	Liquid medium	Media composition (L ⁻¹)
T ₁	Standard <i>Cordyceps</i> liquid medium	Potato infusion (prepared from 200 g fresh potato), 20 g dextrose, 2 g KH ₂ PO ₄ , 1 g MgSO ₄ ·7H ₂ O, 1 g Ammonium citrate [(NH ₄) ₃ C ₆ H ₅ O ₇], 5 g peptone, 20 mg Vitamin B ₁
T ₂	Jaggery broth	Jaggery (100 g)
T ₃	Jaggery-Peptone broth	Jaggery (100 g), peptone (5 g)
T ₄	Jaggery-Yolk broth	Jaggery (100 g), egg yolk (10 g)
T ₅	Jaggery-Dextrose-Peptone-Yolk broth	Jaggery (100 g), dextrose (30 g), peptone (5 g), egg yolk (10 g)
T ₆	Sabouraud Dextrose Broth	Sabouraud Dextrose Broth powder (30 g)
T ₇	Yeast Malt extract Broth	Yeast extract (3 g), malt extract (3 g), peptone (5 g), dextrose (10 g)
T ₈	Malt Extract Broth	Malt extract (20 g)
T ₉	Peptone-Yeast Extract broth	Dextrose (20 g), peptone (20 g), yeast extract (10 g)

Table 2: Composition and estimated raw material cost of liquid culture media for submerged liquid spawn production of *Cordyceps militaris*.

Treatment	Medium	Ingredients	Quantity	Estimated cost (₹)	Total cost (₹ per litre)
T ₁	Standard <i>Cordyceps</i> liquid medium	Potato	200 g	8.00	77.00
		Dextrose	20 g	21.00	
		Peptone	5 g	36.50	
		Ammonium citrate	1 g	2.70	
		KH ₂ PO ₄	2 g	4.20	
		MgSO ₄ ·7H ₂ O	1 g	2.60	
		Vitamin B ₁	20 mg	2.00	
T ₂	Jaggery broth	Jaggery	100 g	15.00	15.00
T ₃	Jaggery-Peptone broth	Jaggery	100 g	15.00	51.50
		Peptone	5 g	36.50	
T ₄	Jaggery-Yolk broth	Jaggery	100 g	15.00	19.50
		Egg yolk	10 g	4.50	
T ₅	Jaggery-Dextrose-Peptone-Yolk broth	Jaggery	100 g	15.00	105.40
		Dextrose	30 g	30.12	
		Peptone	5 g	36.50	
		Egg yolk	10 g	4.50	
T ₆	Sabouraud Dextrose Broth	Sabouraud Dextrose	30g	196.8	196.80
T ₇	Yeast Malt Extract Broth	Yeast extract	3 g	14.15	83.90
		Malt extract	3 g	23.19	
		Peptone	5 g	36.50	
		Dextrose	10 g	10.04	
T ₈	Malt Extract Broth	Malt extract	20 g	154.60	154.60
T ₉	Peptone-Yeast Extract broth	Dextrose	20 g	20.10	212.90
		Peptone	20 g	145.60	
		Yeast extract	10 g	47.20	

Table 3: Effect of different liquid culture media on pellet volume of *Cordyceps militaris* after 14 days of submerged cultivation.

Treatment	Liquid medium	Pellet volume (ml)
T ₁	Standard <i>Cordyceps</i> liquid medium	0.917 ^d
T ₂	Jaggery broth	2.500 ^b
T ₃	Jaggery-Peptone broth	4.750 ^a
T ₄	Jaggery-Yolk broth	0.300 ^e
T ₅	Jaggery-Dextrose-Peptone-Yolk broth	1.000 ^d
T ₆	Sabouraud Dextrose Broth	0.500 ^e
T ₇	Yeast Malt Extract Broth	1.333 ^c
T ₈	Malt Extract Broth	2.667 ^b
T ₉	Peptone-Yeast Extract Broth	0.250 ^e
SEm (±)		0.096
SEd (±)		0.136
CD (P = 0.05)		0.286

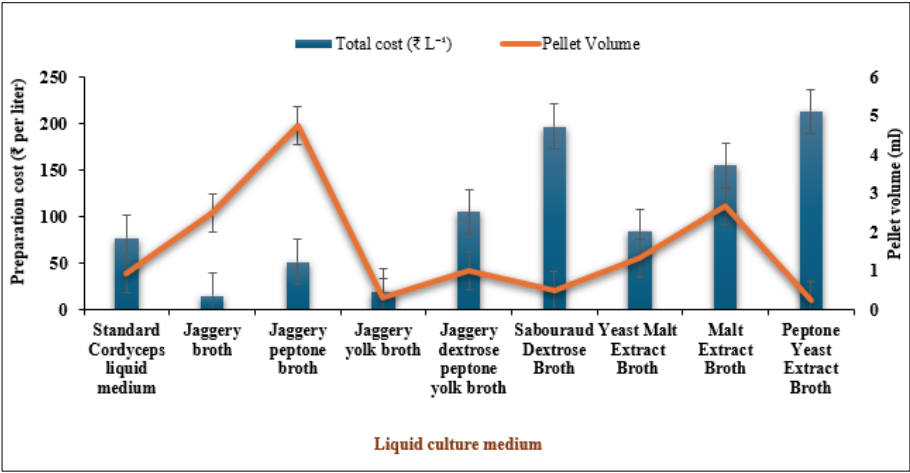


Fig 2: Relationship between preparation cost and pellet volume of different liquid culture media used for submerged spawn production of *Cordyceps militaris*.

Table 4: Effect of different liquid culture media on optical density (OD₆₀₀) of *Cordyceps militaris* after 14 days of submerged cultivation.

Treatments	liquid growth medium	OD ₆₀₀
T ₁	Standard <i>Cordyceps</i> liquid medium	2.473 ^b
T ₂	Jaggery broth	1.610 ^{de}
T ₃	Jaggery-Peptone broth	2.527 ^{ab}
T ₄	Jaggery-Yolk broth	1.497 ^e
T ₅	Jaggery-Dextrose-Peptone-Yolk broth	2.628 ^a
T ₆	Sabouraud Dextrose Broth	1.655 ^{de}
T ₇	Yeast Malt Extract Broth	2.111 ^c
T ₈	Malt Extract Broth	2.378 ^b
T ₉	Peptone-Yeast Extract Broth	1.804 ^d
	SEm (±)	0.050
	SEd (±)	0.071
	CD (P = 0.05)	0.150

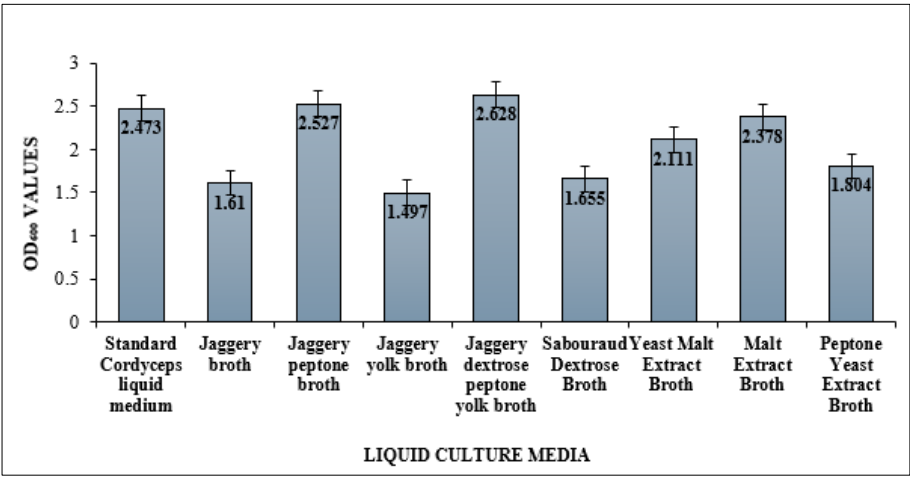
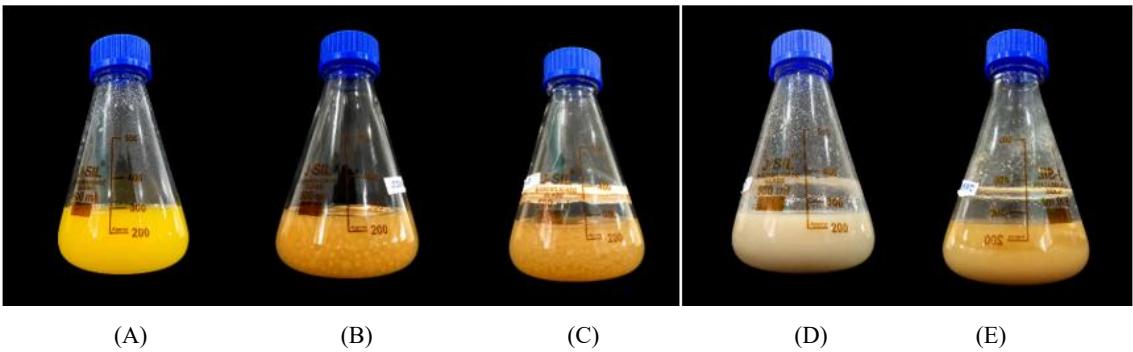


Fig 3: Effect of different liquid culture media on optical density (OD₆₀₀) of *Cordyceps militaris*.



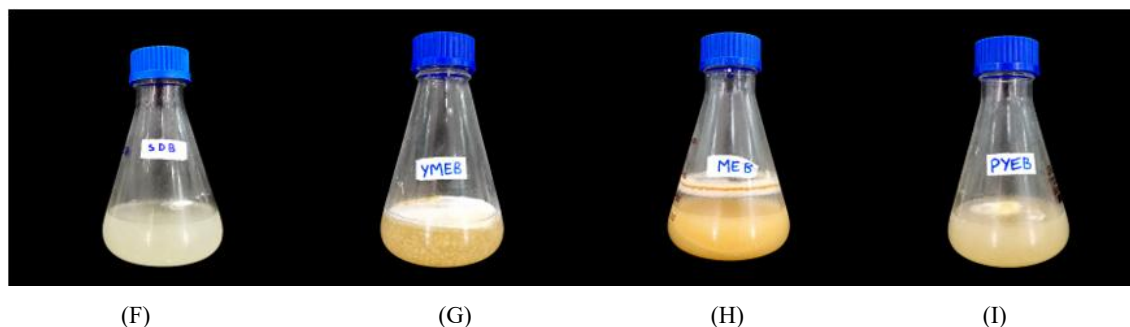


Fig 4: *Cordyceps militaris* grown in different liquid culture media. (A) Standard cordyceps liquid medium (B) Jaggery broth (C) Jaggery peptone broth (D) Jaggery yolk broth (E) Jaggery dextrose peptone yolk broth (F) Sabouraud dextrose broth (G) Yeast malt extract broth (H) Malt extract broth (I) Peptone yeast extract broth

Conclusion

The present study which has been done very first time in Marathwada region during the year 2025- 2026 demonstrated that the composition of liquid culture media significantly influenced the quality of *Cordyceps militaris* liquid spawn produced under submerged cultivation, as reflected by differences in pellet volume and optical density. Among the nine media evaluated, Jaggery-Peptone broth proved to be the most suitable for liquid spawn production, recording the highest pellet volume while requiring a lower preparation cost than the standard *Cordyceps* liquid medium. Although Jaggery Dextrose-Peptone-Yolk broth produced the highest optical density, Jaggery-Peptone broth provided the best overall combination of pellet development, mycelial growth and comparatively lower preparation cost. The findings indicate that an appropriate balance of carbon and organic nitrogen sources is more important than the complexity or cost of the medium in producing high-quality liquid spawn. Owing to its simple formulation, ease of preparation and favourable biological performance, Jaggery-Peptone broth may serve as a cost effective alternative medium for routine laboratory-scale production of *C. militaris* liquid spawn intended for subsequent solid-state cultivation. However, further studies on substrate colonization efficiency, fruiting body yield, bioactive metabolite production and large scale technoeconomic feasibility are required before its commercial application can be strongly recommended.

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