Annals of Phytomedicine 14(2): 700-708, 2025

DOI: http://dx.doi.org/10.54085/ap.2025.14.2.70

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Effect of different solid substrate (grains) and broth medium on yield and biomass production of pharmaceutical important fungus (*Cordyceps militaris* L.)

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Article Info

Article history
Received 26 August 2025
Revised 25 September 2025
Accepted 26 September 2025
Published Online 30 December 2025

Keywords
Cordyceps militaris L.
Cordycepin
Mushrooms
Biological control agent
Radial growth
Mycelium dry weight and Yield

Abstract

Around the world, numerous mushrooms are possessing medicinal properties, and *Cordyceps militaris* L. is a notable entomopathogenic fungus with pharmaceutical importance due to the presence of an antibiotic cordycepin. This compound contributes to its status as one of the most expensive mushrooms due to its high demand in the pharmaceutical industry globally. *C. militaris* naturally grows only on insects, but now it can be artificially cultivated on solid substrates such as grains. The present study was conducted on the artificial cultivation of *C. militaris* using solid substrates like wheat, maize, sorghum, bajra, brown rice and white rice, for this purpose, while seven different broths media were used to radial growth and mycelium dry weight, respectively. Among the solid substrate, bajra and sorghum were found to be superior in terms of yield and height of fruiting body. The highest number of fruiting bodies were observed in sorghum grains (35.00 fruiting bodies), followed by brown rice (30.75 fruiting bodies), wheat grains (27.00 fruiting bodies), bajra grains (26.75 fruiting bodies), white rice (26.25 fruiting bodies), maize grains (12.50 fruiting bodies) in decreasing order across the respective treatments. Sorghum grains provide a nutrient-rich substrate that supports for rapid mycelial growth and fruiting. Among the seven different media and broths, sorghum extract agar media was found to be the best for redial growth in six out of seven media, while maize dextrose broth was observed to be superior for mycelium dry weight in six out of seven broths media.

1. Introduction

Several fungi parasitize on insects, are important biological control agents known as entomopathogenic fungi (EPF). The term entomopathogenic refers to microbes (bacteria, fungi and viruses) capable of infecting insects and completing part of their life cycle within the host (Delgado *et al*., 2011; Chen and Wu 1990). These microbes help decrease insect populations to level at which they cannot cause economic damage to crops and play a role in manage or decrease disease vectors (Mora *et al*., 2017).

Entomopathogenic fungi are commonly in forests and the organic layer of soil. Their application on forests soil surface helps control different insect like pine moth (Gedminas *et al*., 2015). Three genus of entomopathogenic fungi like *Beauveria bassiana, C. militaris* and *Metarhizium* spp. have independently evolved and use proteases and chitinases to infect insects **(**Xiao *et al.,* 2012**).** Chitinases hydrolyze the β-1, 4 linkage of chitin polymer (main exoskeletons component of invertebrates), producing predominant chemicals N, N-diacetylchitobiose. This process breaks down N-acetyl glucosamine (Glc-NAc) monomer from chitobiose (Lu *et al.,* 2005).

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These enzymes, combination with proteases, degrade the insect's cuticle and are linked with different stages of the fungal life cycle, including spores germination, hyphal growth, morphogenesis, nutrition and defence against competitors (Adams, 2004; Shrestha *et al.,* 2016; Shama *et al.,* 2025).

Nowaday's, more than 171 fungal-based products of *Beauveria brongniartii*, *Beauveria bassiana*, *Isaria fumosorosea* and *Metarhizium anisopliae* have been identified and used as biocontrol agents worldwide (Mora *et al.,* 2017). These entomopathogenic fungi belong to two different orders in the kingdom fungi entomophthorales (Phylum: Entomopathoromycota) and Hypocreales (Phylum: Ascomycota) and are found whole world (Augustyniuk-Kram and Kram, 2012; Shrestha *et al.,* 2016**).**

Cordyceps is an important genus in the family Cordycipitaceae, Hypocreales order (Lin *et al.,* 2010). All species of genus *Cordyceps* are entomopathogenic (endoparasitoid), infecting insects from different orders such as Araneae, Blattodea, Coleoptera, Hymenoptera, Diptera, Mantodea, Dermaptera, Hemiptera, Lepidoptera, Orthoptera, Odonata, Phasmatodea. Majority of species infect larvae, adults or pupae of Lepidopter (moths, butterflies) and Coleoptera (beetles), while a few species attack on spiders, fly, ant, bee, wasp, grasshopper, locust, cricket, cicada, bug, scale-insect, coccid, cockroach and termite, mantis, earwig, dragonfly and stick-insect (Quandt *et al.*, 2014; Lu *et al.,* 2017).

C. militarys is an important species of this genus, mostly attack on larvae and pupal stage of 22 families of Lepidopteran order insects

(Gedminas *et al.,* 2015). The fungus colonized its host through mycelium, and spores produce a germ tube after germination, penetrate the cuticle of host **(**Gedminas *et al.,* 2015**).** *C. militaris* in 1997 first time was detected in southern pine forests of Lithuania, where the larvae of pine moth *Dendrolimus pini* L. were found to be sensitive to infection. This species is ecologically and economically important and can be used as myco-biocontrol agent in the humid climate areas to control insect pests in ecofriendly manner (Lu *et al.,* 2017; Ortiz-Urquiza *et al.,* 2015**).** *Cordyceps militaris* fungus has high economic value in pharmaceutical industry and medical science due to presence of bioactive compounds like cordycepin (3'-deoxyadenosine, $C_{10}H_{13}N_5O_3$), ergosterol ($C_{28}H_{44}O$), adenosine ($C_{10}H_{13}N_5O_4$), D-mannitol (cordycepic acid), polysaccharides (Lee *et al.,* 2017). It has long been used as valued traditional Chinese medicines (TCMs) under the name bei-chong-cao or northern worm grass (Chiua *et al.,* 2016).

Cordycepin is a nucleoside antibiotics and major bioactive metabolite of *C. militaris,* it has multiple pharmacological actions, like, immunological regulation (Kiran *et al.*, 2019; Noh *et al.,* 2009), antidiabetic (Cheng *et al.,* 2012; Yu *et al.,* 2015), anticancer (De Silva *et al.,* 2012a), anti-inflammatory (Rao *et al.,* 2010), antitumor (Lin and Chiang 2008; Pao *et al.,* 2012), Apoptotic (Chou *et al.,* 2014; Yang *et al.,* 2012), antiasthma (Hsu *et al.,* 2008), antioxidative (Ramesh *et al.,* 2012), hepatoprotective or antihepatotoxicity (Wang *et al.,* 2012), antivirus, antifungus (Kim *et al.,* 2002), antihyperlipidemia (De Silva *et al.,* 2012b; Guo *et al.,* 2010), antileukemia (De Silva *et al.,* 2013; Thomadaki *et al.,* 2008), improve hormones and sperm motility (Chang *et al.,* 2008), improve sperm production (Lin *et al.,* 2007), improve growth and mineralization (Lin *et al.,* 2015) and due to its high medicinal value, the fungus known as soft gold, biological gold, himalayan gold (Shrestha *et al.,* 2016).

Different techniques have been developed for the artificial cultivation of *C. militaris,* including spawn culture, husked rice culture and saw dust culture (storage/stock culture, pre-culture, popular/ indigenous culture) and, shaking culture, submerged culture, surface liquid culture and continuous/repeated batch culture (special/laboratory culture) for commercial scale production of *C. militaris* (Das *et al.,* 2010). Kobayasi (1941) first succeeded in producing fruiting bodies using rice medium as substrate (10 g rice and 25 ml distilled water in 100 ml Erlenmeyer flask) under *in vitro* condition (Kim *et al.,* 2010**).** For the commercial production of *C. militaris* a simple liquid inoculum method was developed on rice medium. The present study aim to identify locally available superior substrates for commercial production of *C. militaris* fruiting bodies under *in vitro* conditions and to examine the effects of different media on its mycelial growth.

2. Material and Methods

2.1 Experimental site

The experiments were conducted in the Mushroom Laboratory, Department of Plant Pathology, S. V. P. University of Agriculture and Technology, Meerut, Uttar Pradesh, India, during 2018-2021. Geographically, Meerut district is situated between 29°01'N latitude and 77°45'E longitude at an altitude of 237 meters above mean sea level. The district falls under the northwestern plains sub-region of the Upper Gangetic plains. The climate of Meerut is semi-arid and subtropical, characterized by very hot summers and cold winters. The average annual rainfall is 863 mm, 75-80 % of which is received during the southwest monsoon from July to September. Occasional showers may also occur during the winter and summer seasons.

2.2 Chemicals and equipments

Potato dextrose agar (PDA) and yeast extract, glucose/ dextrose, peptone, KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$, NaCl and vitamin B_1 were used. LED lights (500-1,000 lux) and BOD incubator shaker and other equipments were employed in liquid spawn production. All glassware, including flasks and petri plates were sourced from Borosil Glass Works Limited, Mumbai.

2.3 Establishment of seed/pure culture

The culture of *C. militaris* was provided by mushroom laboratory of S. V. P. University of Agriculture and Technology, Meerut, Uttar Pradesh, India and maintained using single hyphal tip method on potato dextrose agar medium (PDA). Single branched hyphae from the periphery of the growing colony were identified under low power (10x) in a compound microscope and transferred to PDA slants. Tubes were stored at 5-10°C and sub culture" at every three months (Dlamini *et al.,* 2012).

2.4 Liquid spawn

Liquid spawn, the first step in artificial fruiting body production of *C. militaris* on solid substrate, was prepared using potato dextrose broth (PD broth) supplemented with glucose/dextrose (1.5%), peptone (0.05%), KH_2PO_4 (0.3 %), yeast extract (0.05%), $MgSO_4.7H_2O$ (0.05 %) and NaCl (0.05 %) in 100 ml distilled water. 20 % PD broth with these chemicals was taken in a 250 ml flask, pH adjusted to 6.0 - 6.5, autoclave at 121°C for 20 min. After cooling, flasks were inoculated with 9 mm PDA discs of 7 days old pure culture of *C. militaris* and incubated at 23-25°C in a BOD Incubator Shaker (150-80 RPM) for 7 days under dark condition (Wen *et al.,* 2014; Wen *et al.,* 2017).

2.5 Solid substrate media preparation

For the fruiting body production of *C. militaris* six types of grains (wheat, maize, sorghum, bajra, brown rice and white rice) use "as solid substrate" (20 g each) with 32 ml of basal media solution (glucose 1.5 g, peptone 0.05 g, yeast 0.05 g, $MgSO_4.7H_2O$ 0.05 g, K_2HPO_4 0.1 g, NaCl 0.05 g and vitamin B_1 0.01 mg with 100 ml distilled water) into a 300 ml bottle. The pH was adjusted to 6.5-7.0 and autoclaved at 121°C for 30 min. After cooling, each bottle was inoculated with 5 ml liquid spawn of *C. militaris* under aseptic conditions and incubated bottles at 22°C ± 2°C for 10-12 days under dark condition for vegetative growth with three replications per treatment. Once the substrate were covered with mycelium, bottles were kept at low temperature 4°C for 24 h subsequently; then bottles were transferred under LED light (500-1,000 lux) with 12:12 h light and dark cycle at 20°C for fruiting body production. Parameters recorded included days for spawn run (DFSR), days for pinhead initiation (DFPI), length of fruiting body (LFB), number of fruiting bodies/bottle (NOFB), and yield (g/ 20 g dry substrate) after harvesting 80-90 days of inoculation.

2.6 Preparation of various media for radial growth and broth for mycelium dry weight

Seven mediums were tested for radial growth potato dextrose agar (PDA), wheat extract agar, oat extract agar, maize extract agar, barley extract agar, bajra extract agar and sorghum extract agar media were used. Grain extracts were prepared by washing of 200 g grains (wheat,

oat, maize, barley, bajra, and sorghum), soaked for 8 h, boiled for 20 min and filtering through muslin cloth, and makeup the volume to 500 ml. 20 g agar was dissolved in 500 ml distilled water and mixed with grain extract, adjusting the total volume to 1000 ml. PDA medium was prepared by dissolving 39 g PDA in 1000 ml double distilled water. All seven media were sterilized at 121°C for 20 min. The sterilized media were poured into petri plates after cooling; petri plates were inoculated with 7 days old *C. militaris* culture of under aseptic conditions, and incubated at 23°C ± 2°C in B.O.D Incubators unit three replications per treatment. Radial growth was measured every 72 h until full petri plate covered by fungus mycelium.

Seven different broths were tested for mycelium dry weight; six grain dextrose broths (wheat, oat, maize, barley, bajra, and sorghum) and PD broth. Broth were dispensed in 50 ml portions into 250 ml conical flask, sterilized at 121°C, 15 lbs psi for 20 min by autoclave and allowed to cool, and inoculated with 9 mm culture discs. The 9 mm disc was cut by cork borer from the periphery. The flasks were incubated at 23°C ± 2°C in BOD units with three replications per treatment. Mycelium was filtered using Whatman filter paper No. 1 and dried at 60°C for 48 h with an weighed an electronic balance after 10 days of inoculation.

2.7 Statistical analysis

A complete randomized design (CRD) was employed, with data collected in three replications. Statistical analyses were conducted using OPSTAT online tools, operating on Windows 7 and Microsoft Office Excel 2007. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at 5% level of significance to compare treatments.

3. Results

3.1 Effect of solid substrate on yield of *C. militaris*

C. militaris has high economic value due the its medicinal properties. Artificial cultivation on solid substrate can improve farmer's financial status. The present study for the artificial fruiting body production of *C. militaris,* six different grains (wheat, maize, sorghum, bajra, brown rice and white rice) were used as solid substrate and different parameters such as days for spawn run (mycelial dilation days in substrate), days for pinhead initiation, length of fruiting body, number of fruiting bodies and yield were recorded and data presented in Table 1, Figures 1 and 4.

Figure 1: Effects of different substrates (grains) on yield of *C. militaris*. 1. Wheat 2. Maize 3. Sorghum 4. Bajra 4. Brown rice 5. White rice.

Maximum yield (15.50 g/ 20 g substrate) was observed in bajra grains, which was significantly similar to sorghum grains (13.70 g/ 20 g grains) and wheat grains (12.95 g/ 20 g substrate) and significantly higher than other treatments. Brown rice followed with 11.32 g/ 20 g substrate. The minimum yield was observed in maize (2.95 g/ 20 g substrate) which was significantly lower than all other treatments. The highest number of fruiting bodies (35.00) was observed in sorghum grains which was significantly higher than other treatments. It was followed by brown rice (30.75 fruiting bodies) which was significantly similar to wheat grains (27.00 fruiting bodies). The minimum number of fruiting bodies (12.50) observed in maize grains which was significantly lower than all other treatments. The highest length of fruiting body (5.02 cm) was observed in sorghum grains and brown rice which was significantly similar with wheat grains (4.25 cm) and white rice (4.15 cm). The minimum length of fruiting body (2.02 cm) observed in maize grains which was significantly lower than all other treatments. The minimum days for spawn run (17.75 days) were observed in wheat grains which were significantly at par with white rice (18.00 days) and brown rice (18.25 days). The maximum days for spawn run (22.25 days) were observed in maize grains. The minimum days for pinhead initiation (20.25 days) were observed in white rice which was significantly at par with brown rice (20.75 days). The maximum days for spawn run (27.00 days) and maximum days for harvesting (90.00 days) were observed in maize grains which were significantly higher than other grains (Table1). The minimum days for harvesting (78.00 days) were white rice which significantly lowers than all other grains.

Table 1: Effects of different substrates (grains) on yield of *C. militaris*

Treatments	DFSR	DFPI	DFH	LFB	NOFB (Per Bottle)	Yield (g/ 20 g grains)
Wheat	17.75	26.50	83.50	4.25	27.00	12.95
Maize	22.25	27.00	90.00	2.02	12.50	2.95
Sorghum	21.75	26.75	85.00	5.02	35.00	13.70
Bajra	20.75	22.50	82.75	3.72	26.75	15.50
Brown rice	18.50	20.75	80.00	5.02	30.75	11.32
White rice	18.00	20.25	78.00	4.15	26.25	10.59
CD at 5%	2.59	2.69	0.68	0.93	3.75	3.78
SE(m)	0.86	0.89	0.28	0.31	1.25	1.26

*Average of three replications

DFSR= Days for spawn run, DFPI = Days for pinhead initiation, DFH = Days for harvesting, LFB = length of fruiting body, NOFB= Number of fruiting body.

3.2 Different liquid media and broths culture

Seven different media and broth were tested for radial growth and mycelium dry weight of *C. militaris,* observation were recorded on 3rd, 6th and 9th days as shown in Table 2, Figures 2 and 3. The results exposed that the maximum radial growth (88.66 mm) was obtain in sorghum grain extract agar media with 9.85 mm/day growth rate on 9th day which was considerably higher than other media. It was followed by wheat extract agar media (83.66 mm with 9.29 mm/day growth rate) on 9th day. The lowest radial growth (65.67 mm) of the *C. militaris* was recorded in oat extract agar media with 8.07 mm/ day growth rate on 9th day which was significantly similar with potato dextrose agar media (73.66 mm with 8.18 mm/day growth rate) achieve lower than all other treatments in Figure 5.

Table 2: Effect of different media on radial growth (mm) and broths on mycelium dry weight of *C. militaris*

Treatment	3rd day	Radial growth rate (mm/day)	6th day	Radial growth rate (mm/day)	9th day	Radial growth rate (mm/ day)	Dry weight in broth (g/ 50 ml)
Wheat extract agar media	33.33	11.11	68.66	11.44	83.66	9.29	1.824
Maize extract agar media	28.33	9.44	61.00	10.16	78.33	8.70	4.214
Barley extract agar media	28.00	9.33	66.66	11.11	79.00	8.77	1.355
Oat extract agar media	32.00	10.66	64.66	10.77	72.66	8.07	2.926
Sorghum extract agar media	39.33	13.11	77.66	12.93	88.66	9.85	1.543
Bajra extract agar media	31.00	10.33	64.33	10.72	79.66	8.85	1.888
Potato dextrose agar media	20.00	6.66	53.00	8.83	73.66	8.18	1.497
CD at 5 %	5.47	3.76	2.91				
SE(m)	1.78	1.22	0.95				

*Average of three replications

In the study of mycelium dry weight, maximum mycelium dry weight (4.214 g/ 50 ml) was observed in maize dextrose broth, which was followed by oat dextrose broth media (2.926 g/ 50 ml) while minimum mycelium dry weight (1.36 g/ 50 ml) was observed in barley dextrose broth which was followed by potato dextrose (PD) broth with 1.50 g/ 50 ml mycelium dry weight.

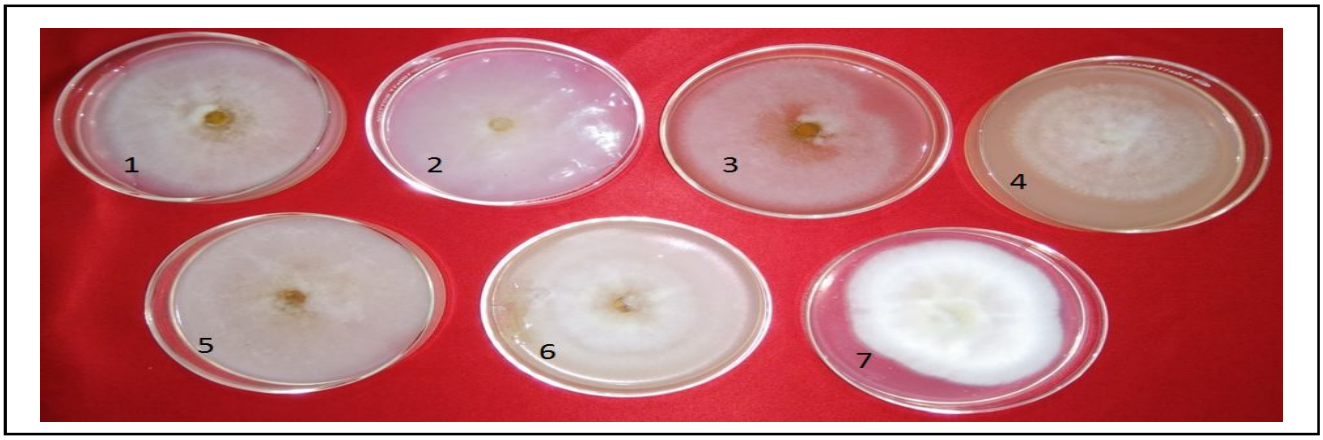

Figure 2: Effect of different media on radial growth (mm) of *C. militaris*. 1. Wheat extract agar media 2. Maize extract agar media 3. Barley extract agar media 4. Oat extract agar media 5. Sorghum extract agar media 6. Bajra extract agar media 7. Potato dextrose agar media.

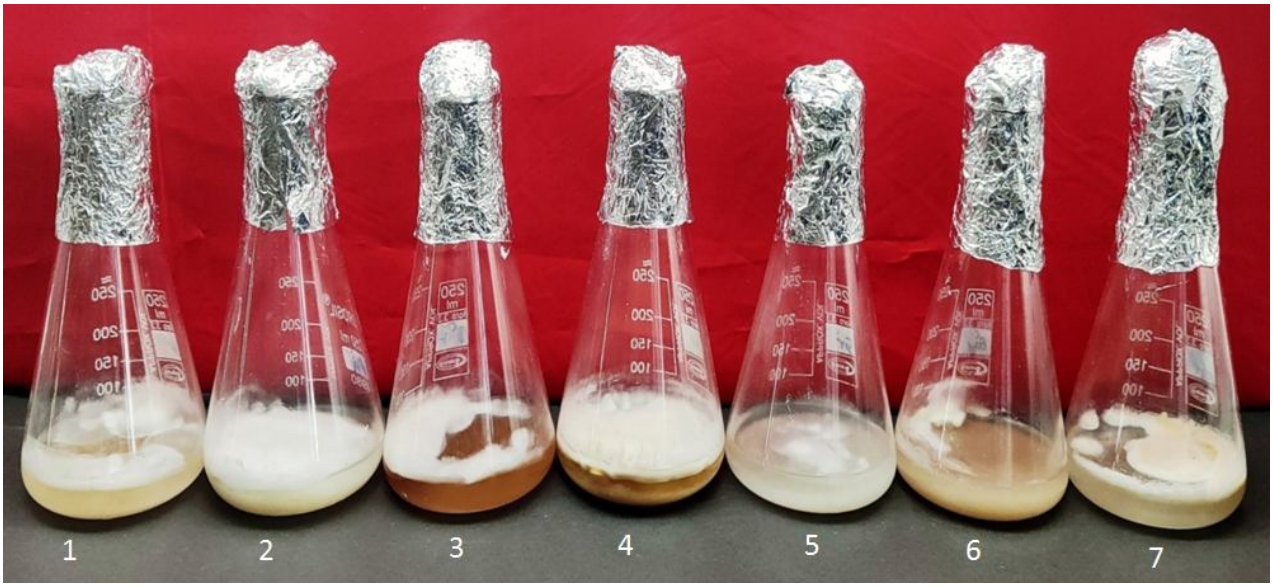

Figure 3: Effect of different broths on mycelium dry weight of *C. militaris*. 1. Wheat extracts broth, 2. Maize extracts broth, 3. Barley extracts broth, 4. Oat extracts broth, 5. Sorghum extracts broth, 6. Bajra extract broth and 7. Potato dextrose broth.

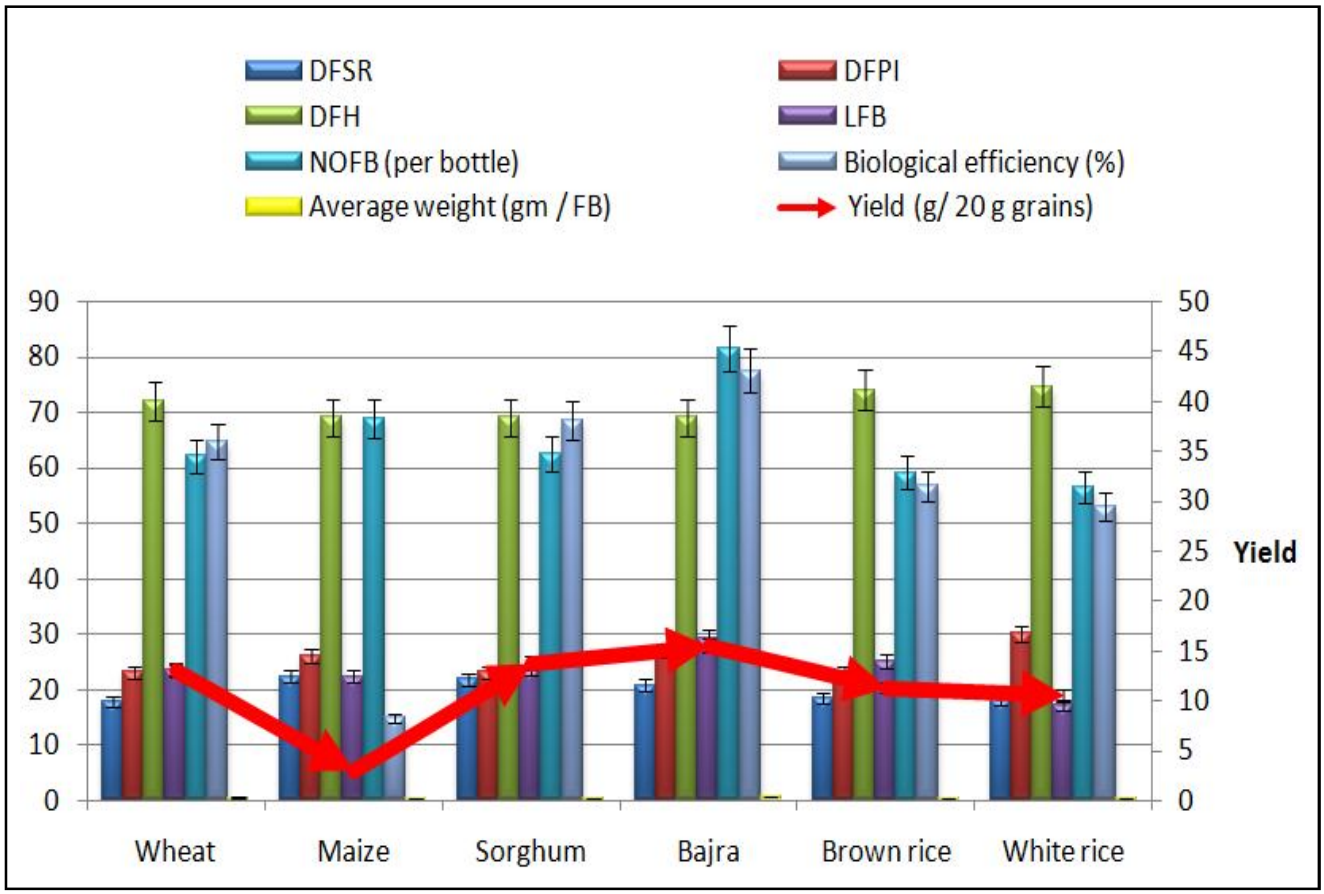

Figure 4: Effects of different substrates (grains) on yield of *C. militaris*.

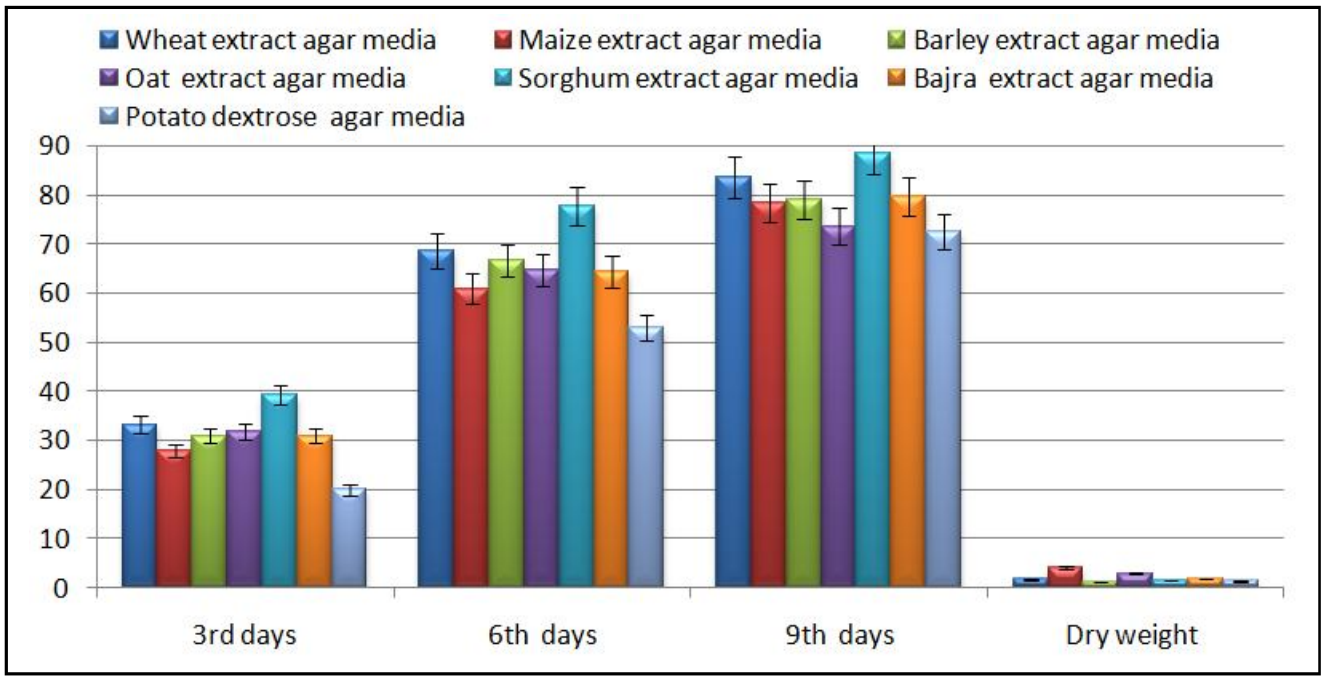

Figure 5: Effect of different media on radial growth (mm) and broths on mycelium dry weight of *C. militaris*.

4. Discussion

4.1 Solid substrate on yield of *C. militaris*

In vitro studies on *C. militaris* began with by De Bary and co-worker in 1887. Nowaday's, interest in artificial cultivation of entomopathogenic fungi has increased due to their bioactive compounds and biocontrol potential (Lin and Chiang, 2008). *C. militaris* has been effectively grown on PDA media (without insects) for commercial high valuable cordycepin production (Shrestha *et al.,* 2012**).**

Solid substrate refers to a cultivation method that utilizes natural substrates contains simple sources of carbon (carbohydrates as energy sources), nitrogen (both inorganic and organic compounds), mineral salts and solid supports. The artificial cultivation of *Cordyceps* primarily involves solid state fermentation (SSF), typically using grain as natural substrate. The grain used as substrate to found the decreasing order of yield on bajra grains (15.50 g/ 20 g substrate) followed by sorghum grains (13.70 g/ 20 g grains), wheat grains (12.95 g/ 20 g substrate), brown rice (11.32 g/ 20 g substrate), white rice (10.59 g/ 20 g substrate), and maize (2.95 g/ 20 g substrate). The significantly higher yield found on bajra grains (15.50 g/ 20 g substrate) and lowest yield found on the maize grains (2.95 g/ 20 g substrate). After soaking all selected grains in the solution, the seeds which become more soften have more growth of myclium on them. Due to the softness of bajra grains, more *C. militaris* yield is obtained. Whereas even after sowing maize grain in the solution, it does not become much softer as grains of bajra. Due to which the myclium of *C. militaris* mushroom is not able to grow well, and which affects the yield of mushroom (Narh *et al.,* 2011; Singh *et al.,* 2021). By optimizing solid substrate fermentation techniques, high-quality fruiting bodies can be attaining to meet the growing demand in the edible fungi market. Meanwhile, to minimize the waste of resources and reduce costs of cultivation, researchers have worked on use of biomass residue as the alternative substrate for the cultivation of *Cordyceps* (Iamtham *et al*., 2022).

The development of *Cordyceps* fruiting bodies is divided into three different stages, *viz.,* mycelium stage, primordial stage, and fruiting body stage. In the mycelium stage, *Cordyceps* require a dark, cold and humid environment, which is favourable for the formation of dense and robust mycelia (Shrestha *et al*., 2006; Wu *et al*., 2016). The highest number of fruiting bodies ware observed in sorghum grains (35.00 fruiting bodies) followed by brown rice (30.75 fruiting bodies), wheat grains (27.00 fruiting bodies), bajra grains (26.75 fruiting bodies), white rice (26.25 fruiting bodies), and maize grains (12.50 fruiting bodies); which are decreasing order in respective treatments. Sorghum grains provide a nutrient-rich substrate that solid support for rapid mycelial growth and fruiting. The sorghum grain has size and higher nutrient content, compared to smaller grains, contribute to faster spawn development and potentially higher yields of fruiting bodies. However, significantly different number of fruiting bodies have been reported when spawns produced with various grains (sorghum, millet, rice, corn and wheat) were used to cultivate *Lentinus subnudus* and *Lentinula sqarrosulus* (Fasidi and Kadiri, 1993; Nwanze *et al*., 2005). In the present observation more or less similar result in *Cordyceps cultivation as* cultivate to *Lentinus subnudus* and *Lentinula sqarrosulus* (Fasidi and Kadiri, 1993; Nwanze *et al.,* 2005).

The highest length of fruiting body (5.02 cm) was observed in sorghum grains and brown rice which was significantly similar with wheat grains (4.25 cm) and white rice (4.15). The minimum length of fruiting body (2.02 cm) observed in maize grains which was significantly lower than all other treatments. These results indicate that the grain, *viz*., sorghum (*Sorghum bicolar*), bajra grains (*Pennisetum glaucum*) white and brown rice (*Oryza sativa*) wheat (*Triticum astivam*), and maize *(Zea mays*) used for *C. militaris* production has a significant effect of length of fruiting body on the

cultivation of mushrooms. Observations from previous studies are in agreement with our findings regarding fruiting body formation in *Cordyceps* (Xiang *et al.,* 2014; Tong *et al.,* 2020; Li *et al.,* 2021). During the fruiting body stage of *Cordyceps*, the primordia develop into mature fruiting structures. The growth and colour change of the fruiting body will be promoted by a certain range of light intensity and controlled photoperiod treatment (Choi *et al*., 2018; Shrestha et al., 2006). Specifically, light intensity and duration of exposure have a great influence on the growth and quality of *Cordyceps* fruiting bodies. The results were found similar with Gao and Wang (2008); Wei and Huang (2009); Kim *et al.* (2010); Shrestha *et al.* (2012); Vega *et al.* (2012).

4.2 Liquid culture and broth medium

3'-deoxyadenosine (cordycepin) is one of the most diverse metabolites produced by *C. militaris,* due to its regulates the broad spectrum of biological activities. Liquid culture offers a simplified cultivation environment and high-level production of cordycepin (Kunhorm *et al*., 2019). In our study, used the various undefined media, *viz*., wheat broths, oat broths, maize broths, barley broths, bajra broths, sorghum broths and potato dextrose broths were evaluated for their suitability in broth culture and biomass production (mycelium dry weight) of *C. militaris.* Among these selected broths, the maize dextrose broth yielded the mycelium dry weight, due to its rich supply of carbon (in the form of starch) as well as organic nitrogen sources (amino acid and protein). Maize dextrose broth provides maximum mycelium dry weight (4.214 g/ 50 ml) followed by oat dextrose broth (2.926 g/ 50 ml), bajra dextrose broth (1.888 g/ 50 ml) H" wheat dextrose broth (1.824 g/ 50 ml), sorghum dextrose broth (1.543 g/ 50 ml), potato dextrose broth (1.497 g/ 50 ml) and barley dextrose broth provide minimum mycelium dry weight (1.355 g/ 50 ml). The maize extract broth was found to produce higher mycelium dry weights due to its rich source of metabolize sugars (glucose and starch), more C:N ratio that supports mycelium development. In compare, sorghum broth contains tannins, and complex carbohydrates, while barley broth contains β-glucans and other antinutritional compounds that can hinder fungal mycelium growth (Kim and Yun, 2005; Wongsa *et al*., 2005; Sehgal and Sagar, 2006; Kumar *et al.,* 2017).

5. Conclusion

C. militaris is an entomopathogenic fungus which naturally growing on insects. Its high medicinal value has increased interest in artificial fruiting body production. For the artificial development of culture and cultivation of *C. militaris* solid substrate play an important role and present study was conducted to find out the effect of six different grains (wheat, oat, maize, barley, bajra, and sorghum) solid substrate in the artificial cultivation of *C. militaris,* out of these grains, bajra and sorghum grains were found better in the reference of yield and height of fruiting body. On sorghum grains indicated the highest fruiting body count, with approximately 180% increase over maize, followed by brown rice (146%), wheat (116%), bajra (114%), and white rice (110%) across treatments. Sorghum extract agar media showed the highest radial growth rate with a 96.85% increase over potato dextrose agar media, followed by wheat (66.86%), oat (60.06%), bajra (55.10%), maize (41.86%), and barley (40.09%) seven different media. Sorghum extract agar media supported the highest radial growth in six out of seven media due to its nutrient rich substrate, while maize dextrose broth yielded the greatest mycelial dry weight in six out of seven broth media.

Acknowledgments

Author would like to thanks, the Head, Department of Plant Pathology, S.V.P.U.A. & T., Meerut, for the authentication of specimens and its culture. We thankful to all technical staff from the Department of Plant Pathology, S.V.P.U.A. & T., Meerut, for providing necessary help during this Ph. D. program.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Satpal Singh, Gopal Singh, Pradeep Kumar, Prashant Mishra, Ramesh Singh, D. V. Singh and Pratiksha Pratap (2025). Effect of different solid substrate (grains) and broth medium on yield and biomass production of pharmaceutical important fungus (*Cordyceps militaris* L.). Ann. Phytomed., 14(2):700-708. http://dx.doi.org/10.54085/ap.2025.14.2.70.**