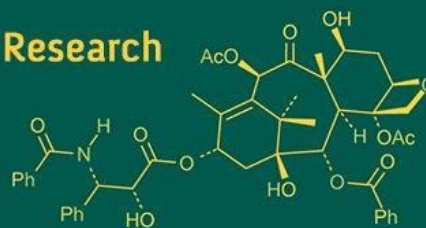


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Evaluation of different pulses extract media on mycelial growth of *Cordyceps militaris* on solid media and in submerged condition for liquid spawn

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Abstract

Cordyceps militaris commonly known as Kirajadi mushroom is a medicinal fungus characterized by its caterpillar-like shape and is classified under the Ascomycetes group of fungi. For centuries, it has been valued as a traditional folk tonic, functional food and natural invigorant in addition to its use as a crude medicinal preparation. This species is renowned for producing cordycepin a potent bioactive compound known to exhibit diverse pharmacological properties including anticancer, antiviral, immune-modulating and anti-infective activities. The colony of *Cordyceps militaris* was circular in shape, densely matted and had a woolly texture. The margin appeared curled with an umbonate and raised elevation. Six different pulses extract agar media viz. pigeon pea extract media, black gram extract media, French bean extract, lentil extract media, pea extract media and potato dextrose agar (PDA) media as control were used to observe mycelial growth (radial growth as well as dry biomass growth) liquid spawn. The maximum mycelial growth and maximum dry biomass was found in pea extract media.

Keywords: *Cordyceps militaris*, pulse extract media, mycelial growth, dry biomass, solid media, submerged culture, liquid spawn

Introduction

The mushroom is an umbrella-shaped fungal fruiting body, or sporophore. In addition to being incredibly nutrient-dense and environmentally friendly, mushrooms provide many health advantages. In nature, mushrooms are frequently found in abundance and present everywhere. Medicinal mushrooms are a special category of fungi known for their therapeutic effects and health-enhancing qualities. These mushrooms contain powerful bioactive substances like beta-glucans, triterpenoids and antioxidants that boost immunity, reduce inflammation and help fight illnesses. One of the largest genera in the Cordycipitaceae family, the genus *Cordyceps* has about 750 variations, making it one of the most diversified. Genus *Cordyceps* have a history for its usage in almost all parts of Asian continent from last thousands of years (Gu *et al.*, 2007) [2]. *Cordyceps militaris* is a common endoparasitoid insect that is found throughout Europe, Asia, North and South America, and normally grows on pupae, however it is less frequently found on larvae. *Cordyceps* has been used to treat lung disease, renal disease, liver disease, cardiovascular disease and excessive cholesterol. It is used to treat immunological disorders as well as chemotherapy (Holliday and Cleaver, 2008; Khan *et al.*, 2010) [3, 4]. Perithecia or flask-shaped fruiting structures are found in the fertile apical region of *C. militaris* club-shaped fruiting body (stromata). Asci that contain ascospores that are distributed for reproduction develop inside these perithecia (Shrestha *et al.*, 2012) [10]. It develops distinctive club-shaped (clavate) fruiting bodies with dark brilliant orange to yellow orange in color and parasitizes insects mainly lepidopteran larvae (Das *et al.*, 2010) [1]. One of its main bioactive ingredients, cordycepin (3'-deoxyadenosine) has a variety of pharmacological properties such as immunostimulatory, antiviral, antifungal, antibacterial and anticancer actions (Paterson, 2008). The advantages of submerged culture include higher mycelial yield in a confined space, faster growth rates, and a reduced risk of contamination. In recent years, *Cordyceps* species are valuable fungi with therapeutic properties and have been widely used for centuries to ensure patient safety

(McKenna *et al.*, 2002; Mehra *et al.*, 2017) [5, 6]. Among the factors influencing fungal growth, culture media composition plays a pivotal role in mycelial development and metabolite biosynthesis (Rahmani *et al.*, 2024) [8, 9]. Pulse-based extracts are rich in proteins, vitamins, and micronutrients, and may provide a low-cost and efficient alternative to conventional media.

Experimental Site

The research work was conducted at the Mushroom Research and Training Centre (MRTC), in Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.). The centre is located on the western side of the Delhi-Dehradun National Highway (NH-58), approximately 10 km north of Meerut city. It lies at 29.074902°N latitude and 77.708568°E longitude.

Establishment of Pure culture

The cultures of *Cordyceps militaris* (Kirajadi Mushroom) were multiplied from the Mushroom Research and Training Centre (MRTC), Sardar Vallabhbhai Patel University of Agriculture and Technology (SVPUAT), Meerut. These cultures were propagated on sterilized Potato Dextrose Agar (PDA) medium in Petri plates. Once sufficient mycelial growth was observed, single-branched hyphae from the advancing edge of the colony were carefully examined under a compound microscope at 10x magnification. The identified hyphae were then transferred aseptically onto PDA slants and Petri plates for culture preservation. These culture tubes and plates were incubated in a BOD incubator maintained at a temperature of 22±2 °C. Subsequently, regular subculturing was performed on PDA medium, and the cultures were preserved under refrigeration at 10±2 °C for further experimental use.

Methods and Materials

To evaluate the mycelial growth and biomass production of *Cordyceps militaris*, Six different pulses based natural media were prepared along with Potato Dextrose Agar (PDA). The legume-based media included extracts from pigeon pea, black gram, French bean, lentil and pea. For each medium, 200 g of the respective legume seeds were thoroughly washed with clean water 2-3 times to remove dust and surface impurities, and then soaked in distilled water for approximately 8 hours. After soaking, the seeds were boiled in 500 ml of distilled water for 20 minutes until the grains softened adequately. Once boiled, the extract was cooled to room temperature and filtered using a double-layered muslin cloth to obtain a clear suspension. The volume of this extract was adjusted to 500 ml with distilled water. Separately, 20 g of agar-agar was melted in another

500 ml of boiling distilled water with continuous stirring. The molten agar was then mixed with the respective legume extracts, and the final volume was brought up to 1000 ml using distilled water. Dextrose (20 g) was also added to each medium as a carbon source. For the preparation of broth (liquid) media, the same procedure was followed without the addition of agar. A 100 ml aliquot of each broth medium was transferred into 250 ml capacity conical flasks for liquid spawn development and dry mycelial weight estimation. For the preparation of broth media, the same procedure was adopted as for their corresponding agar media, except that agar-agar was excluded. In each case, 200 g of legumes (pigeon pea, black gram, French bean, lentil, and pea) were soaked in water for 8 hours, boiled in 500 ml distilled water for 20 minutes, and the extract was filtered through a muslin cloth. The final volume was adjusted to 1000 ml with distilled water and supplemented with 20 g of dextrose. Each broth medium (100 ml) was poured into 250 ml conical flasks. All flasks were plugged with non-absorbent cotton wrapped in butter paper and secured with rubber bands to prevent contamination. The prepared media in both conical flasks and culture tubes were sterilized in an autoclave at 121 °C (15 psi or 1.1 kg/cm²) for 20 minutes. After sterilization, the extract media poured in petri plates. The 9 mm diameter discs were cut by cork borer from the periphery of 7 days old culture of Kirajadi mushroom (*Cordyceps militaris*) and inoculated in the flask and petri plates with the help of inoculation needle. The petri plates and flasks incubated in BOD at suitable temperature in four replications.

Results and Discussion

In this study, *Cordyceps militaris* (locally known as Kirajadi mushroom) was cultivated on various pulse extract-based media to evaluate its mycelial growth under both solid and submerged culture conditions. The solid media included extracts from pigeon pea, black gram, French bean, lentil, and pea, while potato dextrose agar (PDA) served as the control. Radial mycelial growth was measured on the 3rd, 6th, 9th, and 12th day on solid media, and dry biomass was recorded on the 12th day in submerged culture.

As presented in Table 1 and Figure 1, the highest mycelial growth (89.50 mm) on solid media was observed in pea extract agar and a corresponding mycelial growth rate of 7.46 mm/day followed by French bean extract medium with mycelial growth (84.63 mm) with a rate of 7.05 mm/day. In contrast, the lowest mycelial growth was recorded in PDA (73.25 mm) with mycelial growth rate 6.10 mm/day, followed by pigeon pea extract medium (75.50 mm) with mycelial growth rate 6.29 mm/day.

Table 1: Effect of different pulses extracts on mycelial growth of *Cordyceps militaris* on solid media and in submerged condition for liquid spawn

S. No.		Mycelial growth (mm)*				12 th Day growth rate (mm/day)
		3 th Day	6 th Day	9 th Day	12 th Day	
1.	Pigeon pea extract agar media	9.75	35.50	56.25	75.50	6.29
2.	Black gram extract agar media	10.38	39.38	58.38	82.25	6.85
3.	French bean extract agar media	11.00	40.50	60.88	84.63	7.05
4.	Lentil extract agar media	10.00	37.63	56.50	79.25	6.60
5.	Pea extract agar media	11.88	41.75	62.63	89.50	7.46
6.	Potato dextrose agar (control)	9.50	26.38	55.13	73.25	6.10
	CD at 5%	0.81	1.38	1.48	0.63	-
	SE (m)	0.27	0.47	0.5	0.21	-

*Average of four replications

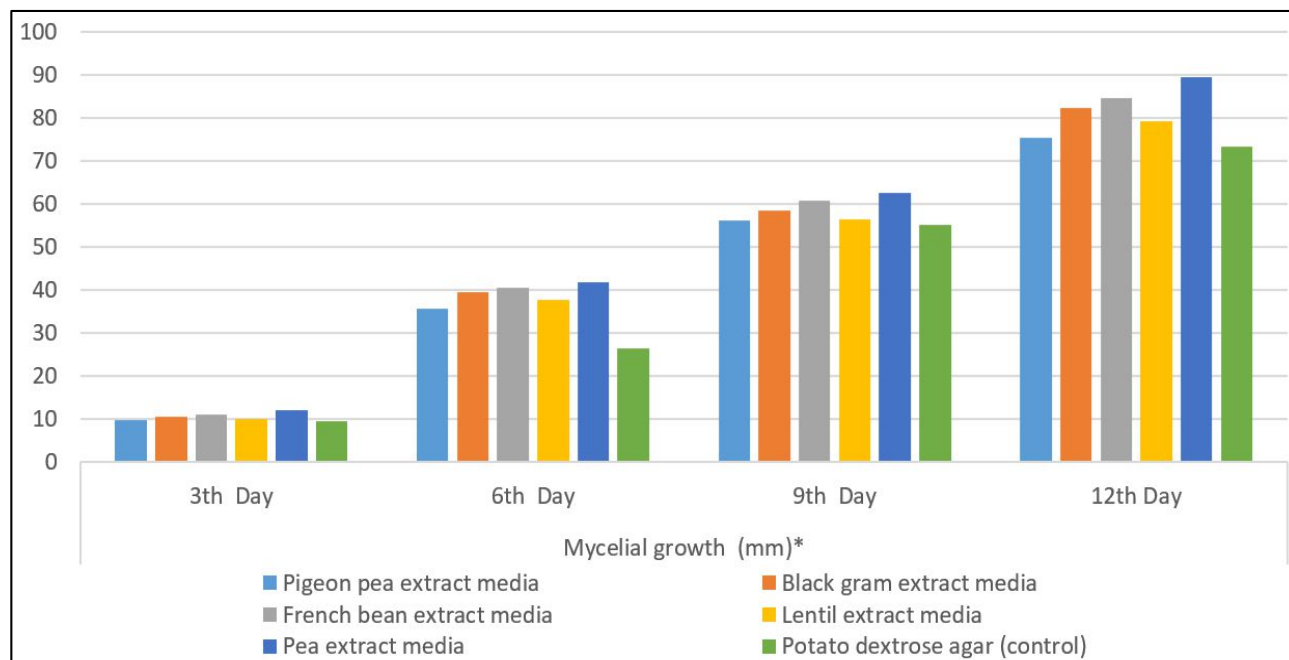


Fig 1: Effect of different pulses extracts on mycelial growth of *Cordyceps militaris* on solid media and in submerged condition for liquid spawn

In Table-02, the maximum dry biomass (1.41 mg/100 ml) was found in pea extract media with biomass growth rate (0.12 mg/day) followed by dry biomass (1.26 mg/100 ml) with biomass growth rate (0.11 mg/day) in French bean extract media. The least dry biomass (0.98 mg/100 ml) in potato dextrose media with biomass growth rate (0.08 mg/day) followed by dry biomass (1.19 mg/100 ml) in pigeon pea extract media with biomass growth rate (0.09 mg/day).

Table 2: Effect of different pulses extracts on mycelial growth of *Cordyceps militaris* on solid media and in submerged condition for liquid spawn

S. No.	Treatments	Dry biomass (mg/100 ml)*	Dry biomass growth rate (mg/day)
1.	Pigeon pea extract agar media	1.19	0.09
2.	Black gram extract agar media	1.24	0.10
3.	French bean extract agar media	1.26	0.11
4.	Lentil extract agar media	1.21	0.06
5.	Pea extract agar media	1.41	0.12
6.	Potato dextrose agar (control)	0.98	0.08
CD at 5%		0.14	-
SE (m)		0.05	-

*Average of four replications

The results were found in aligned with Sung and Spatofoa (2004) [12] who observed the mycelium of *Cordyceps militaris* started developing small nodules, possibly sclerotia, at the center of the medium's surface. These nodules appeared light brown whereas the mycelia at the periphery retained a creamish-white color. Singh (2021) [11] observed that the colony of *Cordyceps militaris* was circular, densely matted, and had a woolly texture with a curled margin. The colony appeared umbonate and raised. The mycelial growth was initially creamish white, but as it

matured, its color transitioned from light brown to orange, while the peripheral mycelia remained creamish white. Rahmani (2024) [8, 9] observed that the mycelium of *Cordyceps militaris* initially appeared cream colored but gradually turned brown as the colony matured, while the peripheral mycelia remained cream-colored, forming a dense, wool-like texture with curled edges in both strains. Under light conditions, the mycelia colour shifted to yellow in strain CM-1 and to yellowish orange in strain CM-3. The results were in accordance with present investigation.

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