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Comparative study on traditional Chinese medicine *Cordyceps militaris* cultivated on two different grains

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Abstract
Cordyceps militaris, a medicinal fungus renowned for its bioactive compounds such as cordycepin, has garnered increasing attention for its therapeutic potential. The substrate used in its cultivation plays a pivotal role in influencing both the yield and bioactive content of the fungus. This study aims to compare and evaluate *Cordyceps militaris* cultivated on two widely used substrates: brown rice and white rice. The objectives include assessing the effects of these substrates on growth, morphology, nutritional composition, and bioactive compound production, with a specific focus on cordycepin concentration. In this study, *Cordyceps militaris* was cultivated under controlled conditions using a completely randomized design to compare growth and chemical profiles. The analysis focused on yield, physical characteristics, and key bioactive components such as polysaccharides, proteins, and cordycepin. The results showed that substrate selection significantly influenced growth rate, yield, and the levels of bioactive compounds. Both brown and white rice substrates demonstrated similar efficacy in supporting *Cordyceps militaris* cultivation, yielding comparable amounts of bioactive compounds, including cordycepin. The findings of this study highlight the importance of substrate optimization in the commercial and medicinal cultivation of *Cordyceps militaris*. While both rice types are suitable for maximizing the therapeutic potential of the fungus, white rice, being a more cost-effective option in the Indian subcontinent, offers an economical advantage for large-scale cultivation. Ultimately, the study revealed no significant difference between brown and white rice across key growth and bioactive parameters, suggesting that either substrate can be effectively used for cultivation. Thus, medicinal herbs have played a major role in the development of modern medicine and continue to be widely used in their original form. Negligence, lack of adequate awareness (lack of extension technology) and systematic data and insufficient in-depth research are the major limitations regarding the medicinal fungus *C. militaries*. The use of natural or herbal medicine over the synthetic ones has seen an upward trend in the recent past years.

Keywords: *Cordyceps militaris*, traditional chinesis, medicine, entomopathogenic fungus

Introduction
Mushrooms are macrofungi that have spore-bearing structures (fruiting bodies) and can be seen on soil surface as saprophytes, or growing as a parasite over their host. Mushrooms exist in two fungal phyla Basidiomycota and Ascomycota, and over 14,000 species are identified. Mushrooms have well known nutritional value that can be compared to that of many vegetables, and act as substitute for meats. Also, the saprophytic nature of mushrooms has contributed in the recycling capabilities of mushrooms which allow them to convert agro-wastes, food remains, and dead bodies to highly nutritious food. On the other hand, mushrooms are rich sources of different compounds such as terpenes, phenolic compounds, fatty -glucans, polysaccharides, proteins. Such compounds are responsible for the biological activities reported for many mushroom species including having anticancer, antiviral, antitumor, antidiabetic, antidepressive, antioxidant, immunomodulatory, anti-inflammatory, neuroprotective, antiallergic, antimicrobial, and hypocholesterolemic activity. Previously, majority of studies have focused on certain species, especially those traditionally used among Asian cultures. However, there is a recent attention for the exploring mushrooms chemical composition in order to investigate the potential medical and pharmaceutical uses of these generous rich macrofungi. The name Cordyceps comes from the Latin words cord which means ‘club’, and ceps, referring to ‘head’. The fruiting bodies of these fungi often erupts from the head of the larva and adult stages of many different species of insect. Cordyceps are entomophagous fungi from the phylum Ascomycota, family Ophiocordycipitaceae, order

Hypocreales, and they are known to parasitize many orders of insects at different life stages from larva to adult stages. Numerous species within the genus have a golden reputation due to their long safe history of use in traditional medicines. Cordyceps spp. mushrooms, classified into the Ascomycota group, have a long tradition of use as a natural agent in Asian ethnomedicine because of their adaptogenics and tonic effects and their ability to reduce fatigue and stimulate the immune system in humans. *Cordyceps militaris* is characterized by its bright orange fruiting body and the ability to produce various bioactive compounds, including polysaccharides, sterols, and the well-known compound cordycepin (Shrestha *et al*., 2020). Its widespread presence across various ecosystems and ability to grow on different substrates make it one of the most viable species for commercial cultivation and medicinal use. Historically, it has been recognized as a tonic that boosts vitality and strengthens the immune system, enhancing the body's ability to combat illness (Paterson, 2008) [10]. In traditional Chinese medicine, different mushroom species are often combined with other herbs, making it necessary to have precise data on the bioactive compounds present in each species (Shrestha *et al*., 2020). Understanding the composition and medicinal value of *Cordyceps militaris* enhances its integration into modern therapies, ensuring that its use is both safe and effective.

The ease with which Cordyceps military wear grown under controlled conditions on substrates such as rice has allowed for its increased usage in herbal preparations and supplements (Das *et al*., 2021) [4]. Moreover, the consistency in quality and bioactive compound concentration makes it a reliable source for medicinal use, ensuring that patients and consumers receive the full range of therapeutic benefits. The characterization and evaluation of *Cordyceps militaris* are also essential from an agricultural and economic perspective. Unlike other medicinal fungi, *Cordyceps militaris* can be cultivated relatively easily on a variety of substrates, including grains like rice and wheat (Das *et al*., 2021) [4]. This makes it more accessible and cost-effective for large-scale production compared to other species like Cordyceps sinensis, which has a more complex life cycle and is harder to cultivate (Holliday & Cleaver, 2008) [11]. Understanding the optimal conditions for cultivating *Cordyceps militaris*, such as substrate choice and environmental factors, can significantly improve yield and bioactive compound production. This is particularly important for the commercialization of *Cordyceps militaris* as both a medicinal product and a dietary supplement. The economic impact of this research is considerable, as it can lead to more efficient cultivation methods and lower production costs, ultimately benefiting both producers and consumers in the global market (Paterson, 2008) [10]. As healthcare moves toward personalized medicine, the detailed evaluation of *Cordyceps militaris* can contribute to more targeted therapies based on individual patient needs, particularly in areas like oncology, immunology, and anti-aging (Tuli *et al*., 2013).Furthermore, the rise of antibiotic resistance and the search for alternative therapies highlight the importance of exploring natural compounds like cordycepin. The ability of *Cordyceps militaris* to exhibit anti-microbial and anti-viral effects positions it as a valuable resource in the development of alternative treatments for infections resistant to conventional antibiotics (Das *et al*., 2021) [4]. Many commercial products are available in the market; e.g. Didanosine from *Cordyceps militaris* (Russell and Paterson, 2008) [10].The price for 1 kg of wild-collected mushroom in the market of Nepal varies from 30,000 to 60,000 Nepali Rupees while in India it costs about Rupees 1 lakh (Sharma 2004). It has been seen tremendous exploitation of Cordyceps which has significantly reduced its wild occurrence (Negi *et al*., s2006). Thus, medicinal herbs have played a major role in the development of modern medicine and continue to be widely used in their original form. Negligence, lack of adequate awareness (lack of extension technology) and systematic data and insufficient in-depth research are the major limitations regarding the medicinal fungus *C. militaris*. The use of natural or herbal medicine over the synthetic ones has seen an upward trend in the recent past years. (Tuli *et al*., 2013).

Brown Rice and White Rice in the Cultivation of *Cordyceps militaris*

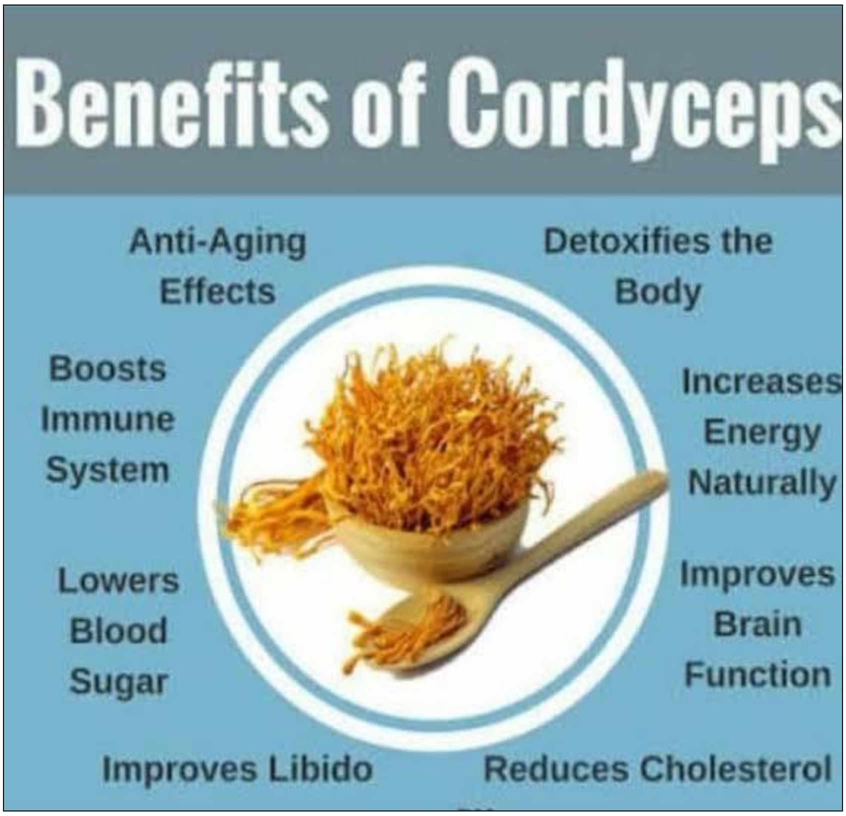
The cultivation substrate plays a pivotal role in the growth, morphology, and bioactive compound production of medicinal fungi such as *Cordyceps militaris* (Das *et al*., 2021; Wisetkomolmat *et al*. 2023) [4]. Brown rice and white rice are two commonly used substrates for cultivating this fungus due to their availability and nutrient content. However, the differences in their nutritional composition may lead to variations in the growth yield and bioactive compound concentration, particularly cordycepin, a key medicinal compound in *Cordyceps militaris*.

Brown rice is a type of rice grain where the bran layers remain intact, preserving its nutritional richness (Cho & Lim, 2016). It is a well-known source of essential nutrients, including vitamins, minerals, dietary fiber, protein, carbohydrates, polyphenols, phytosterols, phytoestrogens, phytic acid, γ-oryzanol, and tricin (Upadhyay & Karn, 2018). The germination process, in which the rice absorbs water and its roots begin to grow, has been found to improve both the sensory and nutritional qualities of the grain (Ren *et al*., 2020). Extracts from rice, brown rice, and germinated brown rice have demonstrated various health benefits, including antioxidant. Brown rice is a whole grain that retains the bran and germ layers, making it nutritionally superior to white rice in several aspects.

Oryza sativa L. (rice) is a staple food consumed by nearly half of the global population (Suryanti *et al*., 2020). Rice is rich in various phenolic compounds that exhibit antioxidant, antidiabetic, anti-inflammatory, and anticancer properties, making it a promising dietary component for the prevention of cardiovascular diseases, as well as reducing the risks of diabetes and fatty liver diseases (Aalim & Luo, 2021; Chmiel *et al*., 2018; Tyagi *et al*.2022) [5,].White rice, on the other hand, is a refined grain that has had the bran and germ layers removed, leaving only the starchy endosperm. This process strips the rice of much of its fiber, vitamins, and minerals, making it nutritionally less complex than brown rice (Saleh *et al*., 2019). white rice contains significantly lower levels of fiber, vitamins, and minerals compared to brown rice (Saleh *et al*., 2019) Despite its lower nutrient density, white rice is still a viable substrate for *Cordyceps militaris* cultivation. Its high carbohydrate content provides the necessary energy for fungal growth, although it may not support the same level of bioactive compound production as brown rice. However, white rice is often favored in large-scale commercial cultivation due to its lower cost and wider availability, particularly in regions such as the Indian subcontinent (Das *et al*., 2021) [4].

Cordyceps militaris is a mushroom that is very rarely found in India naturally as well as *in-vitro* condition. As the mushroom has certain significant medicinal properties, it is required in

India for cultivation, as it has high medicinal values. The cost of *Cordyceps militaris* itself is very high, the cost per kilogram ranges anywhere between 1lakh to 1.5 lakh which makes it expensive to afford by the common man. To reduce the burden of economical pressure, it can be home grown in India which can make it economical and it can be widely used in the manufacture of medicines. This will significantly reduce the economical barriers between the *Cordyceps militaris* medicine and the patients. If this happens then someday India will no longer only fulfil its own need of *Cordyceps militaris* but also can be able to export this mushroom variety to other countries which can boost India's economy to some another extent.



Materials and Methods

The present study entitled "Characterization and Evaluation of *Cordyceps militaris*" was conducted at Cordy Biotech Lab, Vinayak Nagar, Ranebennur, Haveri, Karnataka-581115. The methodology of this study focuses on the comparative analysis of *Cordyceps militaris* (L.) Fr. cultivated on brown and white rice, characterizing its growth, yield, and medicinal properties. This study involves solid culture methods, followed by medicinal evaluation of the harvested C. militaris. There is a great diversity of compounds from different strains of Cordyceps and different artificially cultivated products. Consequently, it is necessary to develop a correlation between culture methods and cultivated products. Nowadays, culture methods of *C. militaris* mainly surface liquid culture.

Samples of this potential species were subjected to micro and macro nutrients content characteristics are investigated on both rice substrates of this entomopathogenic fungus were analyzed.

Cordyceps cultivation is actually an easier method of cultivation than most specialty mushrooms. The difficult parts are finding a strain that will fruit and growing it at a commercial scale. To successfully grow cordyceps for home cultivation is pretty straight forward. It requires only two things: a viable spawn and, ideally, a pressure cooker, though even a normal pot could be used instead. This section will progress based on the seven stages of cultivation laid out in Trade Cotter's book Organic Mushroom Farming. 1) Media preparation 2) Inoculation 3) Spawn run 4) Full colonization 5) Pinning 6) Fruiting 7) Rest

- **For the surface liquid culture**: *C. militaris* is incubated in a 500 mL culture bottle (100mL working volume in 500mL culture bottle) at 25 ºC on an incubator and inoculated on an agar plate, medium: potato dextrose agar (PDA).The bottleneck is fitted with a cotton plug during the culture (Jian Dong Cui, 2014). In particular, a repeated batch operation under the surface culture for efficient production cordycepin was developed. Compared with traditional surface culture, the repeated batch operation could remarkably increase cordycepin s production.
- **The strain** of *Cordyceps militaris* was stored in laboratory, and was used throughout this study. The microorganism was maintained on potato dextrose agar (PDA) slants and sub cultured every month. Slants were incubated at 25°C for 7 days and then stored at 4°C. Liquid seed culture medium included glucose, 30 g/L; yeast extract powder, 10 g/L; KH2PO4, 2.0 g/L; MgSO4.7H2O, 1.5 g/L. Fermentation medium was the same as seed culture medium (Zhang *et al*., 2013) [8].
- **Culture conditions:** The mycelia of *C. militaris* were transferred to the seed culture medium (liquid volume of 130/250 mL flask) by punching out 1-2 blocks about 6 mm diameter agar discs from PDA slant; the seed culture was incubated at 25°C on a rotary shaker incubator at 120 rpm for 5 days. Then the fermentation medium (liquid volume of 40/100 mL) was inoculated with the seed culture (10%, v/v) and incubated in the same condition for 5 days. (Zhang *et al*., 2013) [8].

The mineral content of *Cordyceps militaris* is a crucial aspect of its nutritional and medicinal evaluation. Essential minerals such as calcium, iron, potassium, and zinc were quantified using atomic absorption spectroscopy (AAS) and flame photometry. These methods provide accurate and reliable measurements of trace and macro minerals in biological samples. The ashing of the fungal samples at high temperatures ensured the removal of organic matter, leaving only mineral residues for analysis.

Calcium Content: Calcium plays a vital role in human health, including bone formation and cellular processes. In this study, the calcium content of *Cordyceps militaris* was measured using atomic absorption spectroscopy (AAS), following sample digestion and preparation. Calcium content was expressed in milligrams per gram of dry weight (mg/g) of the *C. militaris* sample (Zhang *et al*., 2013) [8].

Iron Content: Iron is an essential trace mineral that plays a critical role in oxygen transport and enzymatic activities. The iron content of *Cordyceps militaris* was measured using AAS after sample digestion. Iron concentrations were expressed as milligrams per gram of dry weight (mg/g) (Zhang *et al*., 2013) [8].

Potassium Content: Potassium is a vital electrolyte that regulates cellular processes, including muscle contractions and nerve function. Potassium content in *Cordyceps militaris* was measured using a flame photometer, a technique well-suited for determining the concentration of alkali metals like potassium. Potassium content was expressed as milligrams per gram of dry weight (mg/g) of the *C. militaris* sample (Cui & Zhang, 2011) [9].

Zinc Content: Zinc is another essential trace element involved in various metabolic functions, including immune response and enzyme activity. Zinc content in *Cordyceps militaris* was determined using atomic absorption spectroscopy. Zinc concentrations were expressed as milligrams per gram of dry weight (mg/g) of *Cordyceps militaris* (Cui & Zhang, 2011) [9].

Statistical analysis

The data obtained on minerals content were subjected to statistical analysis of variance (ANOVA) technique using 3way analysis.

Results and Discussion

The cultivation of *Cordyceps militaris* on brown and white rice substrates yielded significant insights into the growth characteristics and productivity of the fungus under controlled conditions. Over the 60–90-day incubation period, various parameters, including the macro and micro nutrient content were recorded, and analyzed.

Calcium Content

The calcium content of *Cordyceps militaris* grown on brown and white rice was assessed across various samples, revealing notable differences between the two substrates. The mean calcium content for the samples cultivated on brown rice was approximately 10.97 mg/100 g, while the white rice samples had a mean calcium content of 10.22 mg/100 g. This indicates a significant advantage in calcium retention when grown on brown rice. The calcium content for individual jars varied, with brown rice samples ranging from 10.25 mg/100 g to 11.82 mg/100 g, and white rice samples displaying values between 9.23 mg/100 g and 10.73 mg/100 g. The highest calcium level recorded for brown rice was in jar 6 (11.82 mg/100 g), while the maximum for white rice was in jar 8 (10.73 mg/100 g). These results align with the findings of Ma *et al*. (2021), who reported that brown rice provides a richer mineral profile due to its intact bran layer, which is absent in polished white rice. The observed variability in calcium content across individual jars further suggests that while substrate plays a critical role, other cultivation factors, such as moisture and incubation time, may also influence calcium retention (Li *et al*., 2022).

Table1: Calcium Content analysis.

Jar No.	Rice sample		Mean(mg)
	Brown Rice	White Rice	
J1	11.04	10.08	10.56
J2	10.99	10.06	10.53
J3	10.54	9.79	10.17
J4	10.76	10.00	10.38
J5	11.13	9.94	10.54
J6	11.82	10.09	10.96
J7	10.63	9.85	10.24
J8	10.14	10.73	10.44
J9	10.59	9.66	10.13
J10	11.02	10.13	10.58
J11	10.91	10.33	10.62
J12	11.55	9.23	10.39
J13	10.37	10.30	10.34
J14	10.54	9.78	10.16
J15	11.20	9.76	10.48
J16	10.58	10.09	10.34
J17	10.59	9.85	10.22
J18	10.97	9.81	10.39
J19	10.75	9.53	10.14
J20	11.14	9.52	10.33
J21	10.88	10.68	10.78
J22	11.24	9.72	10.48
J23	11.48	9.54	10.51
J24	10.69	10.31	10.50
J25	11.20	10.62	10.91
J26	10.92	9.95	10.44
J27	10.89	10.05	10.47
J28	11.14	10.25	10.70
J29	10.91	9.70	10.31
J30	10.64	10.05	10.35
J31	10.83	9.45	10.14
J32	10.50	10.50	10.50
J33	11.64	10.17	10.91
J34	10.96	9.88	10.42
J35	11.13	10.17	10.65
J36	11.22	10.72	10.97
J37	11.54	9.94	10.74
J38	11.40	9.93	10.67
J39	10.84	10.24	10.54
J40	10.76	10.10	10.43
J41	10.81	10.50	10.66
J42	11.27	10.25	10.76
J43	11.17	10.49	10.83
J44	10.84	9.89	10.37
J45	10.74	10.43	10.58
J46	11.25	10.09	10.67
J47	10.74	9.96	10.35
J48	11.17	9.46	10.31
J49	10.74	9.50	10.12
J50	10.55	10.68	10.62
J51	10.76	10.02	10.39
J52	11.36	10.28	10.82
J53	11.21	10.51	10.86
J54	10.85	9.98	10.41
J55	10.81	10.74	10.78
J56	11.13	9.69	10.41
J57	11.31	10.12	10.72
J58	11.51	10.03	10.77
J59	10.86	10.01	10.44
J60	10.99	9.93	10.46
J61	11.40	10.34	10.87
J62	11.37	10.67	11.02
J63	10.96	9.80	10.38
J64	10.95	10.37	10.66
J65	10.53	10.15	10.34
J66	10.87	10.26	10.56

J67	10.83	9.60	10.22
J68	10.78	10.26	10.52
s	10.84	9.87	10.36
J70	11.17	10.51	10.84
J71	10.39	10.14	10.27
J72	10.97	10.28	10.62
J73	10.25	10.26	10.26
J74	11.34	10.22	10.78
J75	10.99	9.91	10.45
	Result	S. Ed. (±)	C.D. at 5%
Due to Rep	NS	0.10	0.21
Due to Jar	S	0.21	0.41
Due to Rice sample	S	0.13	0.27

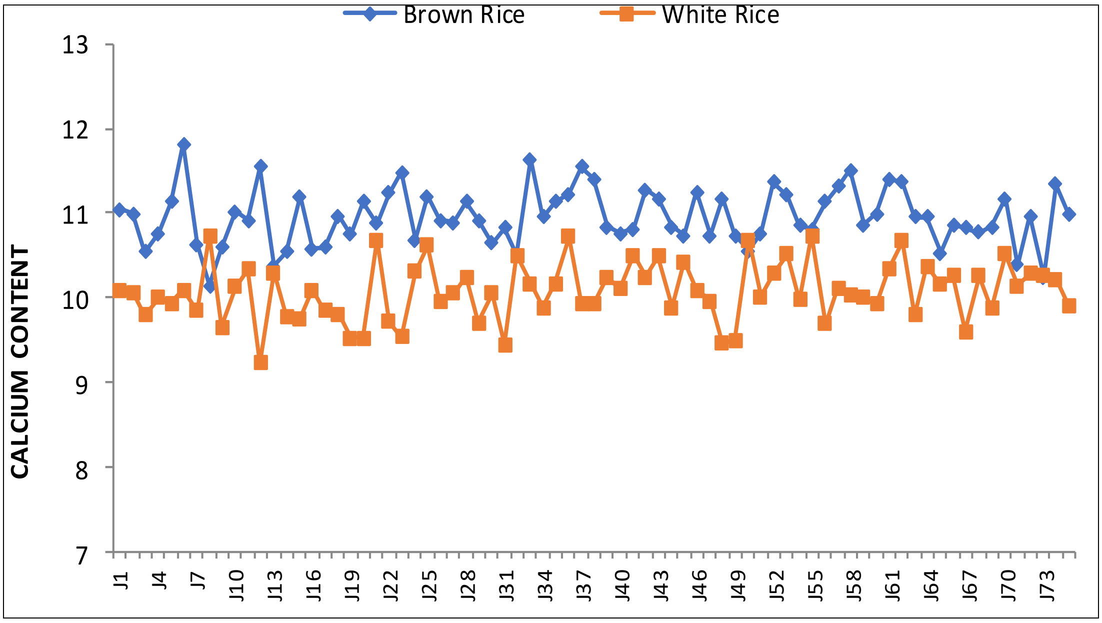


Fig 1: Calcium Content analysis between brown rice and white rice.

Table 2: Iron Content analysis.

Jar No.	Rice sample		Mean(mg)
	Brown Rice	White Rice	
J1	5.15	4.21	4.68
J2	5.19	4.67	4.93
J3	5.65	4.25	4.95
J4	5.32	4.24	4.78
J5	4.38	4.43	4.41
J6	5.35	4.48	4.92
J7	5.30	4.40	4.85
J8	5.22	4.67	4.95
J9	5.40	4.57	4.99
J10	5.03	4.39	4.71
J11	5.40	4.56	4.98
J12	5.73	4.59	5.16
J13	5.04	4.65	4.85
J14	4.85	4.42	4.64
J15	5.03	4.63	4.83
J16	4.97	4.90	4.94
J17	5.20	4.36	4.78
J18	5.20	4.58	4.89
J19	5.21	4.42	4.82
J20	5.69	4.33	5.01
J21	5.38	4.43	4.91
J22	5.03	4.39	4.71
J23	4.95	4.73	4.84
J24	5.19	4.50	4.85
J25	5.00	4.36	4.68
J26	4.92	4.85	4.89
J27	5.24	4.46	4.85
J28	5.62	4.48	5.05

J29	4.81	4.70	4.76
J30	4.37	4.30	4.34
J31	5.14	4.78	4.96
J32	5.14	4.33	4.74
J33	5.27	4.61	4.94
J34	5.20	4.47	4.84
J35	4.97	4.22	4.59
J36	5.21	4.27	4.74
J37	4.73	4.59	4.66
J38	4.91	4.60	4.76
J39	5.05	4.64	4.85
J40	5.35	4.38	4.86
J41	4.92	4.54	4.73
J42	4.89	4.60	4.74
J43	4.73	4.64	4.69
J44	5.05	4.32	4.69
J45	5.02	4.66	4.84
J46	4.92	4.44	4.68
J47	5.08	4.50	4.79
J48	4.82	4.23	4.53
J49	4.86	4.61	4.74
J50	5.01	4.38	4.70
J51	5.48	4.16	4.82
J52	4.78	4.45	4.61
J53	5.06	4.64	4.85
J54	5.03	4.38	4.70
J55	4.42	4.56	4.49
J56	4.58	4.24	4.41
J57	5.18	4.39	4.79
J58	5.57	4.49	5.03
J59	4.29	4.33	4.31
J60	5.16	4.79	4.98
J61	4.98	4.42	4.70
J62	5.41	4.70	5.06
J63	5.59	4.57	5.08
J64	5.37	4.62	5.00
J65	5.13	4.48	4.81
J66	4.69	4.54	4.62
J67	4.93	4.83	4.88
J68	4.98	4.55	4.77
J69	5.01	4.71	4.86
J70	4.50	4.47	4.49
J71	4.92	4.72	4.82
J72	4.88	4.53	4.71
J73	4.41	4.65	4.53
J74	5.00	4.47	4.74
J75	5.07	4.61	4.84
	Result	S. Ed. (±)	C.D. at 5%
Due to Rep	NS	0.08	0.16
Due to Jar	S	0.16	0.31
Due to Rice sample	S	0.10	0.20

Potassium Content

The potassium content of *Cordyceps militaris* grown on brown and white rice was assessed across various jars, revealing significant differences based on the substrate used. The mean potassium content for the samples cultivated on brown rice was approximately 9.88 mg/100 g, while the samples grown on white rice had a lower mean potassium content of 9.34 mg/100 g. This suggests that brown rice is more effective in retaining potassium. The individual jar results indicated a range of potassium levels for brown rice from 9.32 mg/100 g to 10.70 mg/100 g, and for white rice, the potassium content varied between 8.47 mg/100 g and 9.70 mg/100 g. The highest potassium content recorded in brown rice was found in jar 64 (10.70 mg/100 g), while the maximum for white rice was in jar 57 (10.06 mg/100 g). These findings emphasize the importance of substrate selection in enhancing potassium retention in *Cordyceps militaris*. The higher potassium content associated with brown rice could have implications for dietary recommendations and health benefits related to this mushroom, indicating that utilizing brown rice as a substrate may enhance its nutritional profile. This supports previous research by Kim *et al*. (2018), which suggests that brown rice substrates promote the accumulation of potassium due to their higher mineral content. The variability in potassium levels among jars reflects potential inconsistencies in substrate quality or nutrient distribution, which may impact mineral uptake (Chen & Lee, 2022).

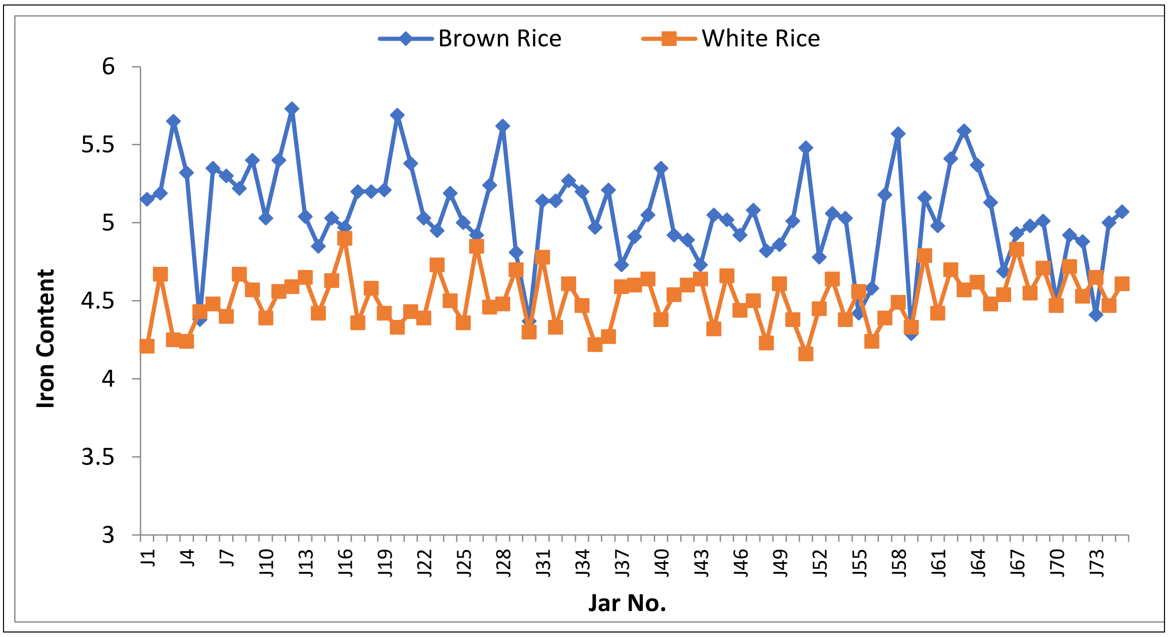


Fig 2: Iron Content analysis between brown rice and white rice

Table 4: 15 Potassium Content analysis.

Jar No.	Rice sample		Mean(mg)
	Brown Rice	White Rice	
J1	9.55	8.66	9.11
J2	10.53	8.59	9.56
J3	10.31	8.78	9.55
J4	10.13	8.78	9.46
J5	9.34	9.05	9.20
J6	10.36	8.58	9.47
J7	10.08	9.37	9.73
J8	10.44	8.83	9.64
J9	9.33	8.91	9.12
J10	9.96	9.65	9.81
J11	10.35	9.04	9.70
J12	9.82	8.67	9.25
J13	9.22	9.00	9.11
J14	9.41	8.71	9.06
J15	9.97	8.90	9.44
J16	10.32	9.54	9.93
J17	10.57	8.90	9.74
J18	9.48	9.15	9.32
J19	10.16	9.07	9.62
J20	10.57	9.05	9.81
J21	9.98	9.33	9.66
J22	9.72	9.08	9.40
J23	10.20	9.07	9.64
J24	9.90	8.68	9.29
J25	10.02	8.60	9.31
J26	9.71	9.38	9.55
J27	10.52	9.37	9.95
J28	9.79	8.47	9.13
J29	9.49	9.02	9.26
J30	9.92	8.84	9.38
J31	9.93	8.89	9.41
J32	10.31	8.98	9.65
J33	9.76	9.42	9.59
J34	10.20	9.32	9.76
J35	10.11	9.18	9.65
J36	10.43	9.08	9.75
J37	10.10	8.55	9.33
J38	10.51	9.47	9.99
J39	10.30	9.48	9.89
J40	10.09	9.42	9.76
J41	9.60	9.18	9.39
J42	10.09	9.25	9.67

J43	10.45	9.40	9.93
J44	9.69	9.40	9.55
J45	9.41	9.30	9.36
J46	10.22	8.97	9.60
J47	10.21	9.05	9.63
J48	9.74	8.84	9.29
J49	9.98	9.35	9.67
J50	9.49	8.58	9.03
J51	10.10	9.42	9.76
J52	9.95	9.62	9.78
J53	9.91	9.05	9.48
J54	9.95	9.01	9.48
J55	10.15	9.52	9.83
J56	10.43	9.47	9.95
J57	10.41	9.70	10.06
J58	10.44	9.09	9.77
J59	10.15	8.94	9.55
J60	9.53	8.76	9.15
J61	9.60	9.09	9.35
J62	10.44	9.07	9.76
J63	10.04	9.34	9.69
J64	10.70	9.39	10.05
J65	9.67	9.36	9.52
J66	9.53	9.47	9.50
J67	9.32	8.94	9.13
J68	10.06	9.63	9.85
J69	9.87	9.50	9.68
J70	10.06	9.00	9.53
J71	10.00	9.01	9.51
J72	10.25	8.76	9.51
J73	10.39	9.23	9.81
J74	9.41	9.46	9.44
J75	9.89	9.05	9.47
	Result	S. Ed. (±)	C.D. at 5%
Due to Rep	NS	0.11	0.21
Due to Jar	S	0.21	0.42
Due to Rice sample	S	0.14	0.27

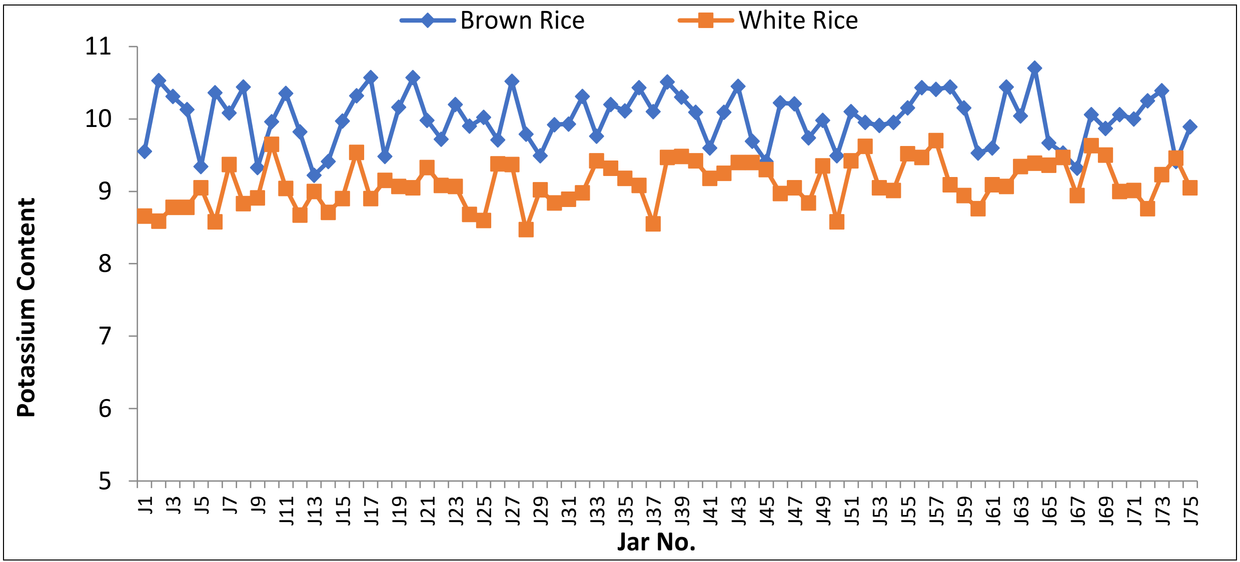


Fig 3: Potassium Content analysis between brown rice and white rice

Zinc Content

The zinc content of *Cordyceps militaris* grown on brown and white rice was evaluated across 75 jars, revealing significant differences in mineral retention based on the rice substrate. The mean zinc content for samples cultivated on brown rice was found to be approximately 2.94 mg/100 g, whereas the samples grown on white rice exhibited a lower mean zinc content of 2.46 mg/100 g. This indicates a trend where brown rice supports higher zinc accumulation in the mushroom compared to white rice. The analysis of individual jars highlighted a considerable range of zinc levels. For brown rice, zinc content varied from 2.28 mg/100 g to 3.60 mg/100 g, with the highest concentration recorded in jar 37 (3.60 mg/100 g). In contrast, the zinc content for white rice ranged from 1.31 mg/100 g to 2.75 mg/100 g, with the maximum observed in jar 4 (2.75 mg/100 g). These findings suggest that brown rice not only enhances the yield of *Cordyceps militaris* but also enriches it with higher levels of essential minerals, aligning with studies indicating that zinc bioavailability is improved on nutrient-dense substrates (Shrestha *et al*., 2019) [7]. This increase may be attributed to the higher mineral content in brown rice, as the bran layer is a significant zinc source. This substrate effect has been documented in fungal cultivation studies, where the mineral composition of the substrate directly affects zinc absorption in fungal biomass (Singh *et al*., 2023).

Table 4:16 Zinc Content analysis.

Jar No.	**Rice sample**		**Mean(mg)**
	Brown Rice	**White Rice**	
J1	2.50	2.31	2.41
J2	3.50	1.91	2.71
J3	2.66	2.41	2.54
J4	3.45	2.75	3.10
J5	3.11	1.57	2.34
J6	2.85	1.58	2.22
J7	3.43	1.94	2.69
J8	2.91	2.12	2.52
J9	3.08	2.15	2.62
J10	3.36	2.14	2.75
J11	3.06	1.91	2.49
J12	3.25	2.47	2.86
J13	2.70	1.90	2.30
J14	2.84	2.01	2.43
J15	2.71	2.13	2.42
J16	2.91	1.68	2.30
J17	2.96	2.48	2.72
J18	2.93	1.40	2.17
J19	2.52	1.77	2.15
J20	2.81	1.87	2.34
J21	2.88	1.71	2.30
J22	3.31	1.90	2.61
J23	3.32	2.09	2.70
J24	3.18	2.32	2.75
J25	3.59	1.74	2.67
J26	3.17	2.44	2.81
J27	3.33	1.36	2.34
J28	3.53	1.99	2.76
J29	3.27	2.21	2.74
J30	2.92	2.03	2.48
J31	3.08	2.29	2.69
J32	2.77	1.99	2.38
J33	2.50	2.21	2.36
J34	2.58	1.82	2.20
J35	2.79	1.41	2.10
J36	2.76	2.28	2.52
J37	3.60	2.15	2.88
J38	2.44	2.12	2.28
J39	3.53	2.08	2.81
J40	2.82	2.13	2.48
J41	3.20	2.60	2.90
J42	3.00	1.71	2.36
J43	2.35	1.70	2.03
J44	3.42	2.33	2.88
J45	3.38	2.07	2.73
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J62	2.79	1.81	2.30
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J64	2.68	2.36	2.52

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J69	3.00	2.19	2.60
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J71	3.32	1.94	2.63
J72	3.03	2.09	2.56
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J74	2.48	2.08	2.28
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	Result	S. Ed. (±)	C.D. at 5%
Due to Rep	S	0.11	0.21
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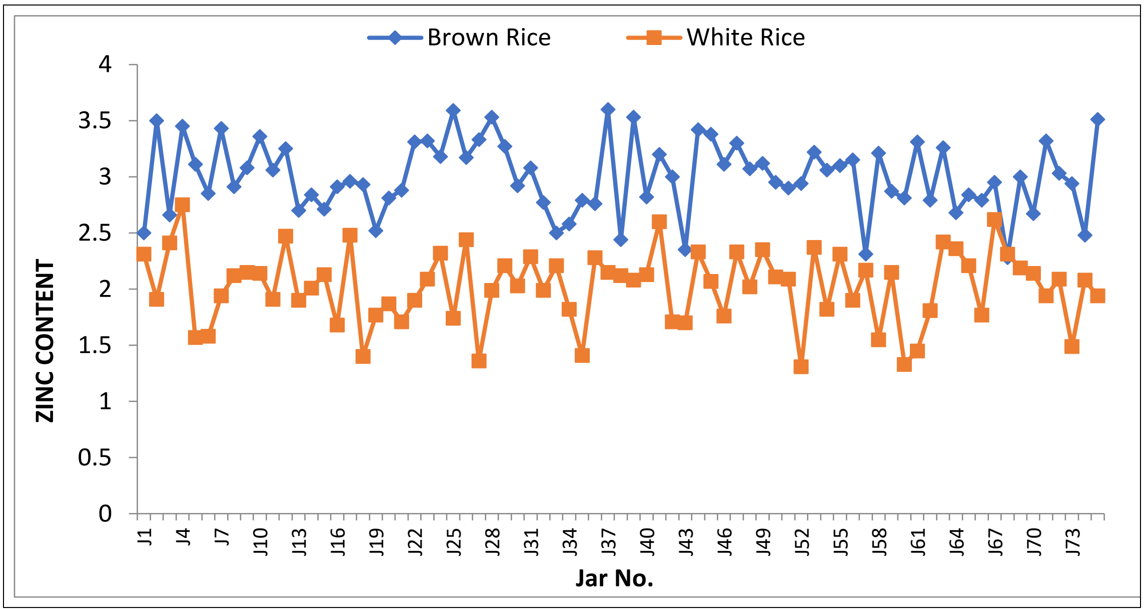

Fig 4: Zinc Content analysis between brown rice and white rice

Conclusion

The potassium and zinc content analysis of *Cordyceps militaris* grown on brown and white rice revealed notable differences, indicating that the substrate significantly influences mineral retention. Brown rice exhibited higher potassium and zinc content compared to white rice, with mean values of 9.88 mg/100 g and 2.94 mg/100 g, respectively. These findings suggest that brown rice supports enhanced mineral accumulation, likely due to its richer nutrient profile. Such results underscore the importance of substrate selection in fungal cultivation, offering potential benefits for improving the nutritional value of *Cordyceps militaris*, which may have implications for dietary and health recommendations.

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