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**Original Research Article**

# Production of Grain Spawn of *Cordyceps militaris*, with the Use of Locale Available Different Grains

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**A B S T R A C T**

Spawn substrate is the important input in the mushroom spawn production. For the production of high quality mushroom spawn with locale available substrate in minimum days is a big dare for mushroom spawn sellers and mushroom growers. The present study was conducted with the aim of finding out the most favorable grain and grains mixture for the production of Cordyceps mushroom grain spawn with locale available grains in minimum days with superior quality. For this study five locally available different grains viz. Wheat, Sorghum, Barley, Bajra, Oat and the mixture of Sorghum, Barley, Bajra and Oat with Wheat grains were used as substrate. The results obtained during the present investigation, Oat grains were found to be the best grains while in case of grain mixture used for spawn production wheat alone was found good for spawn development use as substrate.

**Keywords**

Cordyceps, Spawn, Grains, Mushroom, Wheat, Sorghum, Barley, Bajra and Oat

## Introduction

The early civilizations of the Greeks, Egyptians, Romans, Chinese, and Mexicans appreciated mushrooms as a delicacy, knew something about therapeutic value of mushroom and often used them in religious ceremonies (Chang and Miles 1987). There are at least 12,000 species of fungi that can be considered as mushroom with at least 2000 species showing various degrees of edibility (Chang, 1999). About 300 species have been grown experimentally, 60 cultivated commercially. The majority of these cultivated species are both edible and possess medicinal properties. Out of these, Caterpillar fungus (*Cordyceps* spp.) is the one of major medicinal mushrooms.

The genus Cordyceps is an entomopathogenic fungus group and 750 species in this genus has been recognized (Sung, 1996). *Cordyceps militaris* (an entomopathogenic fungus), is one of them most important medicinal mushrooms, belonging to the class Ascomycetes, has been used popularly as a crude drug and a folk tonic food in East Asia (Ying, 1987). The name comes from the Latin words: *cord* and *ceps*, meaning "club" and "head", respectively. This is name as "soft gold" in China (Winkler, 2008). It possesses many kinds of active components (such as cordycepin, polysaccharides, ergosterol, and mannitol), and due to its several physiological activities, it is currently using

for multiple medicinal purposes (Mizuno, 1999; Song *et al.,* 1998; Nag, 2005). It is widely distributed in North America, South America, Europe and Asia (Mains, 1958), from sub-tropical to temperate regions around the world. The main active constituent of Cordyceps fruiting bodies is cordycepin, which was first extracted from *C. militaris* and then found to be present in *Cordyceps sinensis* (Cunningham *et al.,* 1951) and *Cordyceps kyushuensis* (Ling *et al.,* 2002). Cordycepin has a broad spectrum of biological activity, plays an important role in the treatment of respiratory and cerebrovascular diseases, enhancement of body immunomodulatory function and regulation of liver and renal metabolism (Koh *et al*., 2002; Zhu et al., 1998a, 1998b). Moreover, it also has been used as an anti-cancer, anti-tumor (Pao *et al.,* 2012), anti-fungus (Kim *et al.,* 2002), anti-hyperlipidemia (Guo *et al.,* 2010), antioxidant (Ramesh *et al.,* 2012), and anti leukemia (Thomadaki *et al.,* 2008). Cordycepin is also a Phase I/II clinical stage drug candidate for treatment of refractory Acute Lymphoblastic Leukemia (ALL) patients who express enzyme terminal deoxynucleotidyl transferase (TdT). The natural fruiting bodies of *Cordyceps* are very rare and costly to collect.

Fruiting body production *in vitro* is not repeatable and cordycepin content of natural *Cordyceps* is much lower than that of cultured mycelia (Guo *et al.,* 1998). In recent years *C. militaris* is extensively cultivated in liquid as well as solid media (Das *et al.,* 2010) and is the most successfully cultivated *Cordyceps* species (Kobayashi, 1941; Sung, 1996). Cultivation *of C. militaris* mycelium using artificial media (Masuda *et al.,* 2007) gave higher cordycepin yields. However, only a single *C. militaris* strain was employed and cordycepin production may vary with different strains. The purpose of this study was to examine the consequences of different grains and their mixture with wheat on development of grain spawn of Cordyceps mushroom.

## Materials and Methods

### Experimental site

The experiments were conducted in Mushroom Laboratory Department Plant of Pathology, S. V. P. University of Agriculture and Technology, Meerut, U.P. (India) during year 2018-20, which is situated on the Western side of the Delhi-Dehradun high way (NH-58) at distance of 10.0 km away in the north of Meerut city. The district Meerut is situated between $29^{o}$ 01'N latitude and $77^{o}$ 45'E longitude at an altitude of 237 meters above the mean sea level

### Establishment of pure culture

Culture of *Cordyceps militaries* were purified and maintained by single hyphal tip method. For this purpose, the cultures were grown in sterilized Petri plate on Potato Dextrose Agar Medium (PDA) for 8-10 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10x) in the compound microscope and transferred to PDA slants. These tubes were incubated at 21-24$^{0}$C for about a week, again sub cultured on PDA and then stored in a refrigerator at 5-10$^{0}$C for further use (Dlamini *et al.,* 2012).

### Grain spawn production technology

For this study, the spawn was prepared in half litre capacity wide mouthed glass bottles. The grains were cleaned to remove any broken, shrivelled grains either by

sieving or winnowing or by hand picking of undesired grains. After this, the grains were soaked overnight in clean water and then washed. They were boiled in water for 15 minutes taking care that grains should not split but remain slightly hard after boiling.

The boiled grains were spread in thin layer over a wire net to remove excessive water and enable them to cool about 25-30$^0$C. The cooled grains were then mixed with 1.2 percent commercial grade gypsum ($CaSO_4$) and 0.3 percent calcium carbonate ($CaCO_3$). Gypsum prevents the sticking of wheat grains together and calcium carbonate maintains the pH 5.5 - 7.5. The grains were filled up to (100 mm) in the bottle in three replicates. The bottles were plugged with non-absorbent cotton and covered with butter paper. These bottles were then sterilized at 121$^0$C (15 lbs pressure) for 2 hours on two consecutive days. Sterilized bottles were taken out from the autoclave, while still hot and were shaken to avoid clumping of grains. Sterilized bottles were inoculated by 9 mm disc in individual bottle. The spawn bottles were incubated without shaking at 23±2$^0$C in B.O.D incubator and observations were recorded on 3$^{rd}$, 6$^{th}$ and 9$^{th}$ day till to completely cover by mycelial growth in bottles (Stamets, 2000; Singh *et al.*, 2016).

## Effect of different type of grains on spawn production

In this experiment, different types of grains were used as substrate. For this purpose five grains (i.e. Wheat, Sorghum, Barley, Bajra and oat) were taken. The Spawn was prepared as described above. The grains were filled up to (90 mm) in the bottle in four replicates. The 7 days old culture of *Cordyceps* sp. was inoculated by 9 mm diameter disc in individual bottle under aseptic condition. The spawn bottles were incubated without shaking at 23±2$^0$C in B.O.D incubator and observations were recorded on every three days interval till the first bottle completely covered by mycelial growth in anyone.

## Effect of different grains mixture on spawn production

In this experiment, different types of grains mixture (1:1) were used as substrate. For this purpose four grains i.e. Sorghum, Barley, Bajra and oat were mixed with Wheat grains in equal amount (1:1). The Spawn was prepared as described above. The grains were filled up to (90 mm) in the bottle in four replicates. The 7 days old culture of *Cordyceps sp.* was inoculated by 9 mm diameter disc in individual bottle under aseptic condition. The spawn bottles were incubated without shaking at 23±2$^0$C in B.O.D incubator and observations were recorded on every three days interval till the first bottle completely covered by mycelial growth in anyone.

## Statistical analysis

The suitable statistical design (CRD) was applied and the data thus obtained were analyzed statistically. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at five percent level of significance for comparison with other treatment (Gomez and Gomez, 1984, Kumar *et al.*, 2019).

## Results and Discussions

In the present investigation of different type grains results shows that the maximum mycelial growth (88.25 mm) was obtained on 9$^{th}$ day when oat grains used as substrate with growth rate (9.80mm/days) which was significantly superior to all other grains spawn. It was followed by wheat (84.00

mm) with growth rate (9.33mm/days). The minimum mycelial growth (68.00 mm) with growth rate (7.50mm/days) was observed in Barley which was significantly lower than all other grains and it was followed by the Pearl millet (73.25mm) with 8.13mm/days growth rate on 9$^{th}$ day, results are shown in Table 1.

In the study of different grains mixture (1:1) with wheat grains, were used as substrate. Maximum mycelial growth (88.25 mm) was obtained on 9$^{th}$ day in wheat grains with growth rate (9.80mm/days) it was significantly superior than all other treatments. It was followed by (83.00 mm) in Wheat grain + Sorghum grain (1:1) with growth rate (9.22mm/days). The minimum mycelial growth (69.50 mm) with growth rate (7.72mm/days) was observed in Barley which was significantly similar with Wheat grain + Barley grain (71.50) with growth rate (7.94 mm/days and it was followed by Wheat grain + Pearl millet grain (74.25mm) with 8.25mm/days growth rate on 9$^{th}$ day, results are shown in Table 2.

These results were found in proximity with the research findings of Sharma and Puttoo (2004) reported that barley, corn and oat grains were more efficient than wheat grain. Similarly, Sharma *et al.,* (2003) observed that oat; kutki and maize grains took minimum time for spawn development.

Pathmashini *et al.*, (2008) revealed that the efficacy of four different types of grain spawns viz. kurakkan, maize, sorghum and paddy on oyster (*Pleurotus ostreatus*) mushroom production. Four types of spawns were tested on a medium based on sawdust. Highest mean numbers of sporophore (fruiting bodies) were noticed in the harvests obtained from sorghum spawn (7.67+ or -0.66). The kurakkan spawn significantly. Enhanced biological efficiency and increased size and yield, when compared with other spawn types viz; maize, sorghum, and paddy.

Mbogoh *et al.*, (2011) reported the spawn is pure culture of mycelium growing on a solid substrate such as cereal grain. Maize, wheat and millet grains were used as substrates for production of grain mother spawns of *Pleurotus ostreatus*. Linear mycelium extension was measured. Bhadana (2014) was also reported the best grain mother spawns from the maize substrate being the best, followed by wheat, then millet.

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