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Evaluation of different organic additives effects on spawn production of *Cordyceps militaris*

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Abstract
Spawn is a fundamental element in mushroom production. Present study was conducted to determine the effect of different organic additive on spawn production of *Cordyceps militaris*, for this purpose cereals flour such as Maize, Rice, Sorghum, Barley and Oat flour were used while Chickpea, Pigeon pea, Pea, Black gram and Green gram flour were used as pulses flour for spawn improvement and were added in wheat grains. The results obtained from present investigation show that, in case of cereal flour, maximum spawn growth was found in maize flour while in case of pulse flour use as organic additives, pigeon pea flour was found best organic additive for spawn production.

Keywords: Spawn, organic additive, mushroom, chickpea, pigeon pea, pea, black gram, maize, rice, sorghum, barley and oat

Introduction
Mushrooms are delicious, nutritionally rich, medicinally important and non-conventional sources of human food. Mushroom production is regarded as the second most important commercial microbial technology next to yeast for large scale profitable bioconversion of lignocellulosic waste from agro industry (Chang, 1999) [1]. There are at least 12,000 species of fungi that can be considered as mushrooms with at least 2000 species showing various degrees of edibility. About 300 species have been grown experimentally, 60 cultivated commercially. Majority of these cultivated mushroom species have both edible and medicinal properties. Out of these, Caterpillar fungus (*Cordyceps spp*), which is also known as Kira-jar, is one of major medicinal mushrooms (Cunningham, e*t al*. 1951) [2].
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* and *Cordyceps kyushuensis* (Ling, *et. al.* 2002) [11]. 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 (Zhu et al., 1998a, 1998b) [20, 21]. Moreover, it also has been used as an anti-cancer, anti-tumor, anti-fungal (Kim, *et al*. 2002) [8], anti-hyperlipidemia (Guo, *et al.* 2010) [7], antioxidant (Ramesh, *et al.* 2012) [15], and anti-leukemia (Thomadaki, *et al.* 2008) [19]. Cordycepin is also a Phase I/II clinical stage drug candidate for the 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) [6].
In recent years *C. militaris* is extensively cultivated in liquid as well as solid media (Das, *et al.* 2010) [3] and is the most successfully cultivated *Cordyceps* species. Cultivation of *C. militaris* mycelium using artificial media (Masuda, *et. al.* 2007) [12]. gave higher cordycepin yields. However, only a single *C. militaris* strain was employed and cordycepin production may vary with different strains. Keeping in view the above nutritional and medicinal importance of *C. militaris* mushroom and possibilities cultivating these mushrooms in the rural as well as urban areas of the country, the present investigation was undertaken.

Material 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° 01'N latitude and 77° 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) [4].

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 3rd, 6th and 9th day till to completely cover by mycelial growth in bottles (Stamets, 2000 and Singh, *et al.* 2016) [18, 17].

Effect of different Cereal flour (Organic additives) on spawn growth

In this study, cereals flour (Organic additive) *viz.* Maize, Rice, Sorghum, Barley and Oat flour with @ 5% were mixed as a supplement with wheat grain before sterilization. Spawn was prepared as described previously. The grains were filled up to 90 mm in the bottle in three replicates. The 7 days old culture of Shiitake and Cordyceps were 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 until the first bottle completely covered by mycelial growth in anyone (Rigoberto, *et al.* 2014) [16].

Effect of different Pulse flour (Organic additives) on spawn growth

In this Study, different pulses flour (organic additive) *viz.* Chickpea, Pigeon pea, Pea, Black gram and Green gram flour with @ 5% were mixed as a supplement with wheat grain before sterilization. Spawn was prepared as described previously. The grains were filled up to 90 mm in the bottle in three replicates. The 7 days old culture of Shiitake and Cordyceps were 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 until the first bottle completely covered by mycelial growth in anyone (Singh, 2016) [17].

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) [5, 9, 10].

Result and Discussion

Results of different cereal flour (organic additives) shows that, maximum spawn growth (89.33 mm) was found in maize flour on 9th days with (9.92 mm/day) growth rate which was significantly similar with barley flour (88.33 mm) with (9.81 mm/day) growth rate and it was followed by spawn growth (79.66 mm) found in sorghum flour with (8.85 mm/day) growth rate. The minimum spawn growth of the Caterpillar Fungus (*Cordyceps militaris*) was recorded in wheat grain without organic additive i.e. control (74.00 mm) with (8.22 mm/day) growth rate which was significantly similar to oat flour (75.66 mm) with (8.40 mm/day) growth rate and rice flour (77.00 mm) with (8.55 mm/day) growth rate on 9th days, results are shown in Fig 1.

In the study of different pulse flour (organic additives), maximum spawn growth (89.67 mm) was found in pigeon pea flour on 9th days with (9.70 mm/day) growth rate which was significantly superior to all other treatments and it was followed by spawn growth (84.33 mm) found in green gram flour with (9.33 mm/day) growth rate. The minimum spawn growth of the Caterpillar Fungus (*Cordyceps militaris*) was recorded in wheat grain without organic additive i.e. control (75.00 mm) with (8.33 mm/day) growth rate which was significantly similar to black gram flour (78.00 mm) with (8.67 mm/day) growth rate. It was followed by pea flour (82.00 mm) with (9.11 mm/day) growth rate on 9th days, results are shown in Fig 2.

These results were found in proximity with the research findings of Ramabadran and Eswaran, (2000) [14] had tried the various substrates for spawn production, partially-filled paddy grains (PFPg) and sorghum grains were supplemented with Horse gram flour 3% rapidly colonised by *Pleurotus eous* and it was found to be highly favourable.

Moonmoon, *et al.* (2010) [13] evaluated different levels (10%, 15%, 20%, 25%, 30%, 35% and 40%) of wheat bran (WB), rice bran (RB), maize powder (MP) and their combination (WB+RB+MP = 1:1:1) were used as supplements with the substrate, saw dust (SD) for the cultivation of *Lentinula edodes.* best organic supplement for mycelial growth of *Lentinula edodes* saw dust (SD) supplemented with 25% wheat bran (WB).

Rigoberto, *et al.* (2014) [16] tested different types of spawns of *Lentinus edodes*: Control (C) (millet seed, 100%), F1 (millet seed, 88.5%; wheat bran, 8.8%; peat moss, 1.3%; and CaS04,

1.3%) and F2 (the same formula as F1, but substituting the wheat bran with powdered wheat straw). The mean Biological Efficiency (BE) varied between 66.0% (C-IE-256) and 320.1% (F1-IE-124), with an average per strain of 125.6%. The highest mean BE was observed on spawn F1 (188.3%), significantly different from C and F2.

Singh, *et al.* (2016) [17] evaluated, three different organic additives (pulses flour) on mycelial growth and reported maximum mycelial growth (100.00 mm length) in substrate added with 2% pigeon pea flour on 20th days. The minimum mycelial growth of the *P. djamor* (75.00 mm length) were recorded in the substrate added with 1% black gram flour followed by control (72.33 mm) on 20th days respectively.

Kumar, (2019) [9, 10] reported the maximum growth in Pigeon pea powder @ 1% (90.00 mm), which was followed by Rice powder @ 1% (85.66 mm) and the minimum growth, was observed in control (71.33 mm) in case of Strain CI-17-04. While In case of strain CI-17-08 Maximum growth was observed in Pea powder @ 1% (90.00 mm) which was followed by Maize powder @ 1%, (86.66 mm) and minimum growth was recorded in the control (74.00 mm).

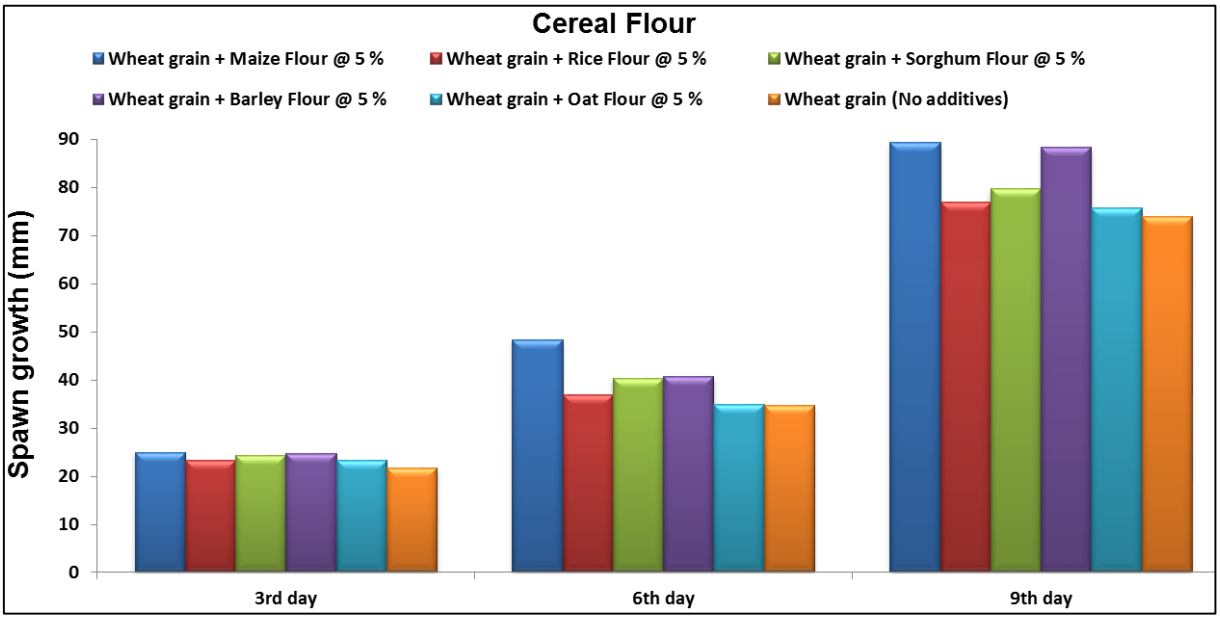

Fig 1: Effect of different Cereal Flour (Organic additive) on spawn growth (mm) of *Cordyceps militaris.*

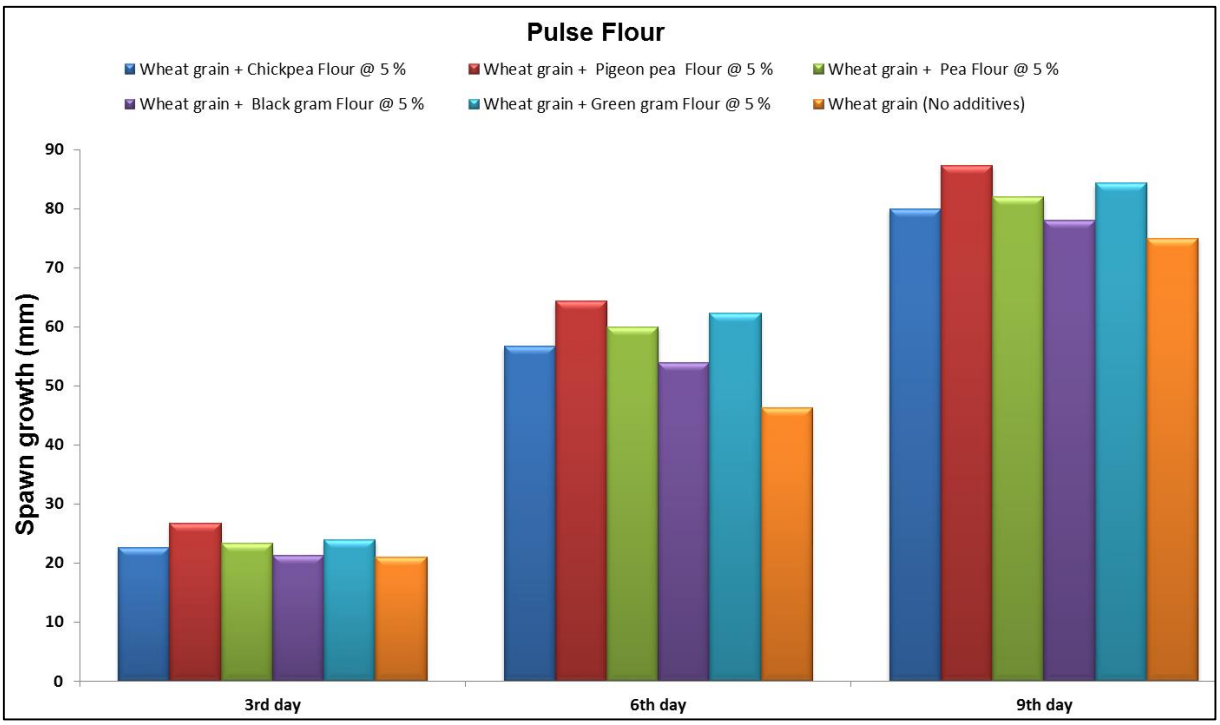

Fig 2: Effect of different Pulse Flour (Organic additive) on spawn growth (mm) of *Cordyceps militaris.*

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