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Research Article

# Equine Dung as an Organic Nitrogen Supplement in Compost Formulation for Cultivation of White Button Mushroom [*Agaricus Bisporus* J.E. Lange (Imbach)]

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**Abstract**

**Purpose:** The present study aimed to utilize the equine dung as an organic nitrogen supplement in commercial compost formulations for the cultivation of white button mushroom.

**Method:** Four different compost formulations (C1 to C4) were prepared using wheat straw or in combination with paddy straw as the basic ligno-cellulosic substrate material. The initial nitrogen levels were maintained at 1.48–1.79% by incorporating various nitrogen enrichment materials such as chicken manure, equine dung, mustard oil cake and wheat bran.

**Results:** The C4 compost formulation (composed of wheat straw – 150 kg, equine dung – 400 kg, mustard oil cake – 30 kg, wheat bran – 30 kg, gypsum – 25 kg, and urea – 6 kg) exhibited the highest biological efficiency of 18.20%, outperforming poultry manure-based compost formulations. Among the various physico-chemical properties of the matured compost, the C:N ratio was identified as a critical factor for achieving higher yields and an extended cropping period.

**Conclusion:** The experimental findings demonstrate an environmentally sustainable approach for utilizing equine dung waste in a productive way to cultivate protein-rich food. This study highlights the potential of equine dung as a valuable component in compost formulations for white button mushroom production.

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**Keywords:** C: N ratio; Mushroom compost; Waste management; Poultry manure

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## 1. Introduction

For thousands of years, mushrooms have been prized as highly nutritious foods by many civilizations in the world. Mushrooms are perhaps the only fungi deliberately and knowingly consumed by human beings and they supplement the human diet with various ingredients not encountered or deficient in food items of plants and animal origin. Mushrooms occupy a major share in rapidly growing new food category products. The nutritional attributes of edible mushrooms and the health-benefiting effects of the bioactive compounds they contain make mushrooms a health food (Gupta et al., 2018; Sharma and Annepu, 2018). The mushroom production has registered manifold increase with the beginning of 21st century (Sharma et al., 2017; Okuda, 2022). Among the cultivated edible mushrooms, the share of *Agaricus bisporus*, popularly known as white button mushroom is significant. The production of all types of mushrooms in India during 2020 was about two lakh Metric tons (MT) and the bulk of it was the white button mushroom (Sharma, 2021). *A. bisporus* is cultivated on a specially prepared substrate called compost which is a product of fermentation by a number of thermophilic organisms that decompose plant residues and other organic and inorganic matters. The main purpose of composting is to release the nutrients of the straw and supplements and to transform them in such a way that they are suitable for supplying nutrition to this mushroom. Compost, if properly prepared, is very selective in nature and only *A. bisporus* mycelium can grow successfully on it at the practical exclusion of other competing organisms.

Agricultural waste materials generally, cereal straws are favored all over the world for the purpose of composting and wheat straw is the most preferred basic raw material for compost preparation. Mushroom growers adopt different proportions of raw materials, depending upon the availability and their cost (Singhal et al., 2019; Jayaraman et al., 2024). In the composting process, poultry manure is considered as a suitable organic nitrogen supplement to increase the biological efficiency and yield potential of *A. bisporus*. It was also reported that addition of poultry manure to the straw adds a significant amount of fine dry matter and improves the texture of compost. From many years, the commercial compost formulae with poultry manure remained as standard for cultivation of *A. bisporus* (Noble et al., 2024). However, in recent times there is an increased concern over the entry of antibiotic residues, antibiotic resistance genes into the food chain through organic manures, especially poultry manure. Several studies have confirmed the movement of multidrug resistance from poultry farms to agriculture farms (Yanget al., 2020a; Shuang et al., 2023). Hence, there is a need to

find suitable alternate nitrogen supplements in composting process for the cultivation of *A. bisporus* mushroom.

Further, Shri Mata Vaishno Devi Shrine, a world-famous pilgrimage located in the Jammu region of India hosts a large population of mules and ponies who are used for carrying the pilgrims from the base camp of the shrine to the main temple. These mules and ponies generate a lot of dung that has become a menace for the local people as well as Shri Mata Vaishno Devi Shrine Board. It was estimated that these mules and ponies are generating about 7 MT of dung per day. Efforts are being made to put this equine dung for use in some productive process. In this series, the present study was carried out to use equine dung as an organic nitrogen supplement in compost formulation and to compare the designed formulation in terms of quality parameters with the formulations already in practice for cultivation of *A. bisporus* mushroom.

## 2. Materials and methods

### 2.1. Source of mushroom culture

Pure culture of *A. bisporus* strain U3 was obtained from the Indian Council of Agriculture Research-Directorate of Mushroom Research, Solan (India). The culture was multiplied and maintained on Malt Extract Agar media and maintained by periodical sub culturing at 22±1°C. Spawn used for cultivation of button mushroom was prepared on wheat grains following Gupta et al. (2020).

### 2.2. Composting Process

Compost was prepared using four different formulations as given in Table 1. The substrates were formulated to maintain the initial nitrogen levels of 1.48-1.79%. Compost formulation I (C1) which is presently being recommended by SKUAST-Jammu and used by the local mushroom growers (Gupta et al., 2017) was kept as the control. The compost was prepared in the composting shed of Mushroom Research and Training Centre of Division of Plant Pathology, SKUAST-Jammu. Composting shed was cleaned thoroughly with water and washed with 2% formalin solution before spreading the wheat straw. Wheat straw (chopped into 3-5 cm size) was spread on the floor up to the height of 9-12 inch and the straw wetted thoroughly by spraying water and turning the straw with the help of iron forks. The straw was kept thoroughly wet for the next 24 hours. After two days equine dung, poultry manure, wheat bran, urea and mustard oil cake were added as per the compost formulation given in Table 1 to the wetted straw and water was sprinkled over them to moisten the mixture. The C1 formulation contains the wheat straw 621.5 g, chicken manure 248.8 g, mustard oil cake 31.1 g, wheat bran 62.1 g, gypsum 31.1 g and urea 5.0 g per each kg of the substrate. The C2 contains wheat straw 248.8 g,

paddy straw 373.8 g, chicken manure 248.8 g, mustard oil cake 31.1 g, wheat bran 62.1 g, gypsum 31.1 g and urea 5.0 g per each kg of the substrate. The C3 contains wheat straw 202.5 g, equine manure 539.7 g, chicken manure 135.6 g, mustard oil cake 40.5 g, wheat bran 40.5 g, gypsum 33.7 g and urea 8.1 g per each of the substrate. Whereas, the C4 formulation contains wheat straw 233.3 g, equine manure 624.3 g, mustard oil cake 46.8 g, wheat bran 46.8 g, gypsum 39.0 g and urea 9.4 g per each kg of the substrate. The wheat straw and other ingredients were mixed thoroughly with the help of iron forks and made into stake (pile) of 5'×5'×5': (length × breadth × height). The pile was kept as such for another six days and the first turning was given on the seventh day. Further six turnings were given on Day 10, Day 13, Day 16, Day 19, Day 22 and Day 25. Gypsum @ 25 kg was added to the pile during the third turning in all the compost formulations. After seven turnings the pile was broken and spread.

**Table 1.** Composition of compost formulations

Ingredient	C <sub>1</sub> (kg)	C <sub>2</sub> (kg)	C <sub>3</sub> (kg)	C <sub>4</sub> (kg)
Wheat straw	500	200	150	150
Rice straw	---	300	---	---
Equine manure	---	---	400	400
Chicken manure	200	200	100	---
Mustard oil cake	25	25	30	30
Wheat bran	50	50	30	30
Gypsum	25	25	25	25
Urea	4	4	6	6
Nitrogen (%)	1.63	1.48	1.79	1.63

### 2.3. Analysis of physico chemical properties

Ingredients as well as finally prepared compost of different formulations were analyzed for various physico-chemical properties *viz.*, moisture content (%), bulk density ( $\text{kg m}^{-3}$ ), porosity (%), pH, electric conductivity (EC) ( $\text{dS m}^{-1}$ ), total carbon (%), total nitrogen (%) and C: N ratio. Moisture content was determined by Hot Air Oven Method (AOAC, 1995). Physical parameters like bulk density and porosity were determined by Keen Raczkowski Box Technique (Keen and Raczkowski, 1921). pH and EC were measured using pH meter and EC Meter (Model EC Tester 11), respectively. Carbon (%) was determined by ignition method (loss on ignition) as described by Nelson and Sommer (1996) and nitrogen was estimated by Kjeldahl method. The C:N ratio of each sample was calculated by deriving ratio of carbon to nitrogen of each sample.

### 2.4. Cropping trials

Once the compost was ready, the pile was broken and the ready compost was spawned @ 0.75% on wet weight basis. Spawned compost was filled in polybags of the size 18×24 inches and they were kept in the cropping room where proper conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), relative humidity (70-80%) and CO<sub>2</sub> (1200-1500 ppm) were maintained by regulating the ventilation and airflow. During the spawn run period, less ventilation was provided to increase the relative humidity in the cropping rooms. Once the spawn run was completed, 1.5 to 2 inches of casing layer comprising of a mixture of 2 years old farm yard manure (FYM), garden soil and sand in the ratio of 4:2:1 was applied to the cropping bags. Watering was done only to keep the casing layer moist. After 7-8 days of casing, pin heads started emerging. At that time, ventilation was provided to reduce the CO<sub>2</sub> content to 1200-1500 ppm and temperature was reduced to 16-18°C. The relative humidity was maintained at 85-90% using the humidifiers. Observations on days to spawn run, days to fruit initiation, number of fruit bodies (per 100 kg of compost), average weight of fruiting body (g), stipe length (cm), pileus diameter (cm), number of flushes and biological efficiency (BE%) (fresh mushroom yield in kg per 100 kg of compost) were recorded during the crop cycle.

### 2.5. Statistical analysis

The cultivation trials were conducted in completely randomized design with five replications for each compost formulation. The linear regression line was drawn, and coefficient of determination ( $R^2$ ) was calculated to test the significance levels between pair of parameters. A multiple linear regression model was developed for mushroom yield as dependent variable and physico-chemical properties of compost as regressed variables. For all experiments one way ANOVA was applied to determine the significance between different treatments using R Studio open-source software (version 1.0.136).

## 3. Results and discussion

In the present study, four different compost formulations were compared by analyzing the physico-chemical properties of various ingredients used in the composting process (Table 2) and the final compost samples (Table 3). The ingredients used in the composting process vary widely in their characteristics which caused variation in physico-chemical characteristics of the composted substrate (Table 2). The experimental results revealed that maximum moisture content was observed in chicken manure (55.56%) and minimum in mustard oil cake (9.94%) (Table 2). Bulk density was found maximum in gypsum ( $776.02 \text{ kg m}^{-3}$ ) while the minimum bulk density

was found in rice straw (104.02 kg m<sup>-3</sup>). Maximum porosity (83.48%) was observed in mustard oil cake while the minimum porosity percentage was observed in gypsum (41.38%). Urea had the maximum pH of 8.07 and minimum pH was observed in mustard oil cake (5.39). Maximum EC of 9.40 dS m<sup>-1</sup> was observed in chicken

manure and minimum was reported in urea (0.52 dS m<sup>-1</sup>). Maximum carbon content was found in equine dung (39.0%) and minimum in urea (20.38%). Maximum nitrogen content was found in urea (46.61%) and minimum nitrogen content was observed in wheat straw (1.02%) (Table 2).

**Table 2.** Physico-chemical properties of the ingredients of compost formulations

Ingredient	Moisture content (%)	Bulk density (kg m <sup>-3</sup> )	Porosity (%)	pH	EC (dS m <sup>-1</sup> )	Carbon (%)	Nitrogen (%)
Wheat straw	16.46	105.60	82.97	6.60	3.40	31.67	1.02
Chicken manure	55.76	513.17	61.67	7.79	9.40	33.00	2.60
Equine manure	33.53	328.83	70.00	7.33	6.20	39.00	1.62
Rice straw	31.69	104.02	75.33	7.44	4.07	34.00	1.03
Mustard oil cake	9.94	483.03	83.48	5.39	3.07	29.12	4.16
Wheat bran	12.15	340.34	65.00	6.19	1.92	33.33	4.07
Gypsum	-	776.02	41.38	7.63	5.20	-	-
Urea	-	720.02	46.63	8.07	0.52	20.38	46.61

Physical and chemical properties of substrates are the prerequisite for providing essential nutritive compounds and adequate texture for mycelial growth in mushroom cultivation (Chang and Miles, 2004; Bellettini et al., 2019; Nandeha et al., 2024). The bio-engineering characteristics of basic ingredients of the compost varied widely and caused variation in composted substrate characteristics and directly affect the properties of compost and mushroom yield (Wakchaure and Singh, 2013; Carrasco et al., 2018). A variety of substrates in composting showed with lot of variation in the yield of button mushroom (Annepu et al., 2024), thereby indicating the relation of physicochemical factors of compost like pH, temperature profile, C:N ratio, moisture content and the compositional changes in the substrate (Kariaga et al., 2012; Yang et al., 2020b).

Physico-chemical characteristics of the compost prepared using different formulations are presented in Table 3. Moisture content is an important factor in composting and the range of moisture content in different compost formulations varied from 60.26–65.67% (Table 3). The results of our experiment indicated that maximum moisture content (65.67%) was found in C<sub>2</sub> which was at par with C<sub>3</sub> (65.37%) while the minimum moisture content of 60.26% was observed in the compost formulation C<sub>1</sub>. Griensven et al. (2008) reported that the optimum moisture at the end of the composting process should be 71–74%, which corresponds to 65–69% at inoculation. Liang et

al. (2003) and Aguilar-Paredes et al. (2023) suggested that moisture content has a greater influence on microbial activity and subsequent maturity of the compost. The significant positive relationship between the moisture content, organic matter of compost and mushroom yield was reported by Sanchez et al. (2008). Bulk density was found to be significantly higher ( $p < 0.05$ ) in C<sub>1</sub> (361.63 kg m<sup>-3</sup>) and minimum in C<sub>4</sub> (356.17 kg m<sup>-3</sup>). Compost as compressible material exhibits both elastic and plastic behavior causing variation in moisture content, bulk densities during composting with respect to height and time of pile. Khater (2015) has reported the bulk density values ranging from 420 to 655 kg m<sup>-3</sup> for different compost types. Wakchaure and Singh (2013) have reported that bulk density of the compost decreased with increase in composting period, however, bulk density increases slightly after addition of gypsum on 10<sup>th</sup> day for both the methods. Pujol et al. (2010) found that bulk density values were between 447 and 502 kg m<sup>-3</sup> for different compost types with varying chemical compositions. It could be seen that the bulk density of compost decreases with increasing compost total organic matter. Wakchaure and Singh (2013) have reported that for all the treatments the initial average substrate bulk density 416 kg m<sup>-3</sup> decreased up to 4 days, increased slightly after second turning (on 8 days) and again decreased to optimal maturity level 342–362 kg m<sup>-3</sup> at the end of composting process. Further, porosity was

found maximum in C<sub>2</sub> (66.96%) while C<sub>3</sub> had the minimum porosity of 58.43%. Porosity of four different composts were found to be statistically non-significant to each other ( $p > 0.05$ ). Khater (2015) reported porosity values ranging from 60.69 to 72.47% for different compost types. The lowest value of the porosity (60.69%) was found for cattle manure compost and the highest value of the porosity

(72.47%) was found for sugar cane plants residues compost.

He observed that the porosity decreased with increasing bulk density and moisture content with the decrease in porosity from 72.47 to 60.69% when the bulk density increased from 420 to 655 kg m<sup>-3</sup> and the moisture content increased from 25.6 to 32.1%.

**Table 3.** Physico-chemical properties of compost based on different formulations

Compost formulation	Moisture content (%)	Bulk density (kg m <sup>-3</sup> )	Porosity (%)	pH	EC (ds/m)	Carbon (%)	Nitrogen (%)	C:N ratio
C <sub>1</sub>	60.26	361.63	66.46	7.01	5.60	39.18	2.17	18.20:1
C <sub>2</sub>	65.67	360.20	66.96	7.29	4.10	40.31	2.41	16.94:1
C <sub>3</sub>	65.37	360.50	58.43	6.96	4.80	33.19	2.11	16.51:1
C <sub>4</sub>	60.40	356.17	60.22	7.04	4.47	47.63	2.68	17.81:1
CD	1.17	0.63	0.79	0.21	0.44	5.93	0.10	-
SE (m)	0.38	0.20	0.26	0.07	0.14	1.96	0.03	-

pH and EC are the temperature sensitive properties which vary with temperature during the composting. In our study, maximum pH (7.29) was found in C<sub>2</sub> while the minimum (6.96) was observed in C<sub>3</sub> (Table 4). pH ranges from 7.3-8.2 was considered ideal for the mushroom compost supplemented with different nitrogen base supplements (Lawrence et al., 2005). The findings of the present study are supported by Khater (2015) who reported pH value ranging from 6.3 to 7.8 for different compost types. Royse et al. (2008) stated that compost containing relatively high pH and no ammonia supports good mycelial growth and thus the acceptable range of pH at the time of spawning should be from 6.5-8.2. Kaur and Khanna (2002) showed the pH range of 6.9-8.3 for different compost formulations. The rise in pH values at the initial stage can be attributed to ammonia released and increase of the substrate temperature by efficient thermophilic microbial metabolism (Labance et al., 2006; Qian et al., 2025). Maintaining the compost pH at 6-7 is crucial to reduce the risk of competitive moulds during the button mushroom cultivation (Kredics et al., 2022). Reduction in pH after eight days was reported due to the addition of gypsum in the second turning. It was probably an addition of high-density (788.7 kg m<sup>-3</sup>) gypsum powder during second turning for lowering the compost pH to neutral. Moreover, addition of gypsum to lower the compost pH, affecting the  $\text{NH}_4^+ = \text{NH}_3 + \text{H}^+$  dissection equilibrium (Misz et al., 2024). Gypsum also benefits to maintain the compost texture, preventing it from becoming too dense during the spawn run stage (Gerrits, 1977). EC is a measure of total water-soluble salt content and it plays an important role in

the production of *A. bisporus*. In the present study, C<sub>1</sub> exhibited high EC values (5.60 dS m<sup>-1</sup>) followed by C<sub>4</sub> (4.47 dS m<sup>-1</sup>). The EC of all the compost formulations were found to be statistically different from each other. Khater (2015) reported that the EC values ranged from 2.6 to 4.1 dS m<sup>-1</sup> for different compost types. Low soluble salt concentration is required to increase both the yield and number of fruit bodies while high salt concentration produce negative effect on yield (Sanchez, 2010). Further, maximum carbon content was found in C<sub>4</sub> (47.63%) and the minimum carbon content was found in C<sub>3</sub> (33.19%).

Ibrahim et al. (2009) while studying physiochemical properties of spent mushroom compost concluded that compost having organic matter content between 40-65% supports good yield and early pin head formation. The best organic matter range for ideal compost was 40 to 60 per cent in the studies conducted by Royse et al. (2008). Maximum nitrogen was recorded in C<sub>4</sub> (2.68%), and the minimum value of nitrogen was observed in C<sub>3</sub> (2.11%). Chang and Miles (1995) stated that for *A. bisporus* mushroom generally an initial nitrogen concentration of about 1.5% is considered normal. In addition, there is an evidence that final concentration of nitrogen tends to be stabilized around 2%, both when using initial concentration of nitrogen at 1.3% or when using an initial concentration 2.5% (Griensven et al., 2008). The maximum C:N ratio was found in C<sub>1</sub> (18.20:1) followed by C<sub>4</sub> (17.81:1) but both the treatments were on par with each other. Demirel et al. (2005) reported that in order to improve the C:N ratio and to accelerate composting process, all substrate formulae need the addition of

nitrogen rich supplements at the onset of composting. Composts with high nitrogen contents generally have low C:N ratios, which in our case were found to be in the range of 16.51:1 and 18.20:1 of the ready compost before spawning. The additional nitrogen provided by the supplement implies an added decrease in the C:N ratio, placing the ratio below the recommended range given its low initial value. [Gimenez et al. \(2017\)](#) reported that the application of supplements to composts with lower N contents would provide an even better yield, without affecting the quality of the mushrooms. The observations regarding the yield parameters of button mushroom grown on different compost formulations are presented in [Table 4](#). Minimum number of days for spawn run was taken by C<sub>4</sub> (18.38) followed by C<sub>1</sub> (18.64) and maximum by C<sub>2</sub> (21.29). [Annepu et al., 2024](#) reported that 26-28 days was required for completion of spawn run and first harvest started after 45 days from the day of spawning. The early fruiting was observed in C<sub>4</sub> (28.45) and the maximum number of days taken for fruit formation was observed in C<sub>2</sub> (35.05). [Gupta et al. \(2004\)](#) have reported that in treatments having the maximum depth of compost there was delayed spawn run by 5-9 days and fruiting by another 2-4 days that resulted in production of different compost depth treatments, the lower compost density took lesser number of days for full run followed by early harvest. In our studies, the maximum number of fruiting bodies were found to be maximum in C<sub>2</sub> (140.21) followed by C<sub>1</sub> (133.33) and minimum in C<sub>3</sub> (110.52). All the treatments were significantly different from each other except C<sub>3</sub> and C<sub>4</sub> which are statistically at par. The results presented in [Table 5](#) indicated that the maximum weight of fruiting body (g) was found in C<sub>4</sub> (17.05 g) followed by C<sub>3</sub> (16.24 g) and minimum was found in C<sub>2</sub> (10.94 g). The weight of fruiting bodies of C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub> and C<sub>4</sub> were significantly different from each other however C<sub>3</sub> and C<sub>4</sub> were found to be at par. In our studies, stipe length was maximum in C<sub>4</sub> (4.05 cm) followed by C<sub>3</sub> (3.95 cm) and minimum C<sub>2</sub> (3.47 cm). Statistically this yield parameter was found to be non-significant for all the compost formulations. It

should be taken into account that the absolute stalk length is commercially and economically important. Longer stalks in combination with a tightly closed cap might positively increase total yield and facilitate mechanical harvesting. A long mushroom stalk may not necessarily be considered a good quality and economically important character ([Barh et al., 2020](#)). These observations also showed that the short stalk mushrooms had a tighter closed cap; thus, their quality should be considered higher as compared to the rest. The pileus diameter was higher in C<sub>4</sub> (3.25 cm) followed by C<sub>3</sub> (2.75 cm). Marketability, performance, and the total yield of the button mushroom are affected by the cap diameter. The cap is the preferred part of the button mushroom; thus, mushrooms with high cap weights and low stalk weights are desirable. The provisions of the Commission Regulation of the European Community state that size of cultivated mushrooms is determined by the maximum cap diameter and the stalk length. Accordingly, the minimum cap diameter must be at least 15 mm for closed, veiled and open mushrooms ([Demirer et al., 2005](#)). Generally, mushrooms are produced in a series of harvests also known as “breaks” or “flushes”. The duration of harvest for each flush can take up to 3-6 days while the average time between each flush is around 6-12 days. Although mushrooms continue to be produced for several weeks, mushroom growers cease harvesting when they are no longer economically viable. The results found in our experiment indicated that the maximum number of flushes were found in C<sub>4</sub> (3.60) and minimum in C<sub>3</sub> (3.10). Statistical analysis showed that the number of flushes of only two treatments (C<sub>1</sub> and C<sub>4</sub>) were found to be significantly different from each other however rest all of the treatments were found to be at par with each other. [Wakchaure and Singh \(2013\)](#) have reported the yield of *A. bisporus* strains S-11 and U3 obtained in three harvests for substrates matured under different composting periods (16 to 28 days). Biological efficiency was found maximum in C<sub>4</sub> (18.20%) which was statistically found at par with C<sub>1</sub> (16.86%) followed by C<sub>3</sub> (16.50%) and minimum yield was observed in C<sub>2</sub>(14.50%).

**Table 4.** Yield parameters of fruit bodies obtained from different compost formulations

Compost formulation	Days to spawn run	Days of fruit initiation	Number of fruiting bodies	Avg. weight of fruiting body (g)	Length of stipe (cm)	Diameter of pileus (cm)	Number of flushes	BE (%)
C1	18.64	30.95	133.33	12.98	3.80	2.63	3.40	16.86
C2	21.29	35.05	140.21	10.94	3.47	2.26	3.50	14.10
C3	19.86	29.50	110.52	16.24	3.95	2.75	3.10	16.50
C4	18.38	28.45	112.50	17.05	4.05	3.25	3.60	18.20
CD	0.28	4.12	9.06	1.16	0.11	0.26	0.29	1.53
SE (m)	0.09	1.36	2.99	0.38	0.03	0.08	0.09	0.50

The Spearman's rank correlation studies clearly indicated that the initial moisture content and pH values of the substrate has significant and positive correlation with the days to spawn run ( $r=0.894$ ;  $0.524$ ) and days to fruit initiation ( $r=0.456$ ;  $0.474$ ) (Table 5). The number of fruit bodies has positively and significantly correlated with the bulk density ( $r=0.520$ ) and the porosity of the substrate ( $r=0.826$ ). Further, total number of flushes which is an indication of the longer cropping period and higher production rate has positively and significantly correlated with the carbon ( $r=0.839$ ), nitrogen ( $r=0.708$ ) and the C:N ratio ( $r=0.751$ ). Similarly, the BE also positively and significantly correlated with the carbon ( $r=0.578$ ), nitrogen ( $r=0.431$ ) and the C:N ratio ( $r=0.545$ ). We also tried to establish the relationship between the physico-chemical properties of the compost and the BE using the multiple linear regression model to find out the most critical characteristics of the compost that influences the yield potential of white button mushroom. The model included BE as dependent variable and moisture, bulk density, porosity, pH, EC, carbon, nitrogen, C:N ratio of the compost as the regressed variables. The correlations between BE and C:N ratio were found to be highly significant with an adjusted R value of 0.9776. Barr and

Parandi (2006) stated that the amount of nitrogen should be sufficient in compost because it not only maintains appropriate C:N ratio in compost but also act as energy suppliers to the microorganisms for proper composting. Andrade et al. (2008) reported optimum C:N for *A. bisporus* compost supplemented with soybean meal and cottonseed meal within the range of 22.7:1. Wakchaure and Singh (2013) have reported that the initial substrate C:N ratio  $34 \pm 0.3:1$  decreased to maturity level of 16.15:1 to 18.70:1 during composting for all the treatments. Further, Sebaaly et al. (2019) reported the quality and proximate composition of the button mushroom in different substrate combinations with the C:N ratio varying from 17:1 to 26:1 and found that the better nutritional quality of the mushrooms at lower C:N values. The decrease in C:N ratio during composting was due to loss of organic carbon contents and corresponding relative increase in nitrogen contents.

The substrate decomposition as reflected by loss of carbon contents varied from 21 to 48% to get matured compost for all treatments piles. Quality control during compost production should ensure adequate chemical and physical properties as well as an adequate degree of stability and maturity.

**Table 5.** Correlation between physico-chemical properties of compost and yield parameters

	Days to spawn run	Days of fruit initiation	Number of fruiting bodies	Avg. weight of fruiting body (g)	Length of stipe (cm)	Diameter of pileus (cm)	Number of flushes	BE (%)
Moisture	0.894**	0.456*	0.161	-0.273	-0.445	-0.586*	-0.193	-0.375
BD	0.377	0.383	0.520*	-0.562	-0.448	-0.725**	-0.283	-0.458**
Porosity	0.301	0.490*	0.826**	-0.890**	-0.800**	-0.568*	0.194	-0.669**
pH	0.524*	0.474*	0.404	-0.431	-0.622**	-0.393	0.066	-0.637**
EC	-0.522*	-0.027	0.174	-0.191	0.153	-0.133	0.265	0.274
Carbon	-0.312	-0.163	0.222	0.067	0.304	0.146	0.839**	0.578**
Nitrogen	-0.176	-0.130	0.008	0.169	0.213	0.314	0.708**	0.431*
C:N ratio	-0.363	-0.118	0.443	-0.110	0.282	-0.120	0.751**	0.545**

The substitution of chicken manure with equine dung in compost preparation for white button mushroom cultivation presents promising potential, particularly under seasonal growing conditions. From a circular economic perspective, utilizing locally available equine waste promotes waste valorization, reduces dependency on commercial poultry farms, and contributes to sustainable agricultural practices. Horse manure, rich in lignocellulosic material and typically mixed with straw bedding, offers a favorable structure for aeration and microbial activity during composting. This can lead to improved compost quality, fostering better mycelial growth and mushroom yield. Furthermore, horse farms

generate significant quantities of manure, often underutilized, making it a readily available input for mushroom growers. Transitioning to equine dung also minimizes bio-security risks associated with chicken manure, such as pathogens or antibiotics residues. While further studies are needed to optimize nutrient balance and decomposition rates, early trials and anecdotal evidence suggest that equine dung-based compost can serve as a viable, eco-friendly alternative. Embracing such sustainable resource loops aligns with regenerative agriculture goals and offers resilience against input supply fluctuations, making it a strategic shift for future-proofing mushroom cultivation systems.

#### 4. Conclusion

From the experimental results, it could be concluded that C<sub>4</sub> compost formulation had biological efficiency of 18.20%, and the study proved that equine dung can be successfully used in the composting process for cultivation of white button mushroom, otherwise which either thrown into Ban Ganga river or destroyed in some other manner. The study demonstrated that an optimum C:N ratio, particularly in compost formulations like C<sub>4</sub>, which had a ratio of 17.8:1, led to higher biological efficiency (18.20%). The C:N ratio plays a crucial role in the compost maturation process, affecting the microbial activity and nutrient availability for mycelial growth. Optimizing the C:N ratio enhances the overall yield by promoting better compost quality and stability. Thus, controlling the C:N ratio is vital for achieving higher yields and better quality in button mushroom cultivation.

##### Authors Contribution

RJ-Date collection: SG- Study conception and design and Draft manuscript preparation; SKA and MG-Analysis and interpretation of results: The results were evaluated by all authors, and the final version of the manuscript was approved.

##### Availability of data and materials:

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

##### Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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