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Comparative evaluation of growth, yield and biological efficiency of selected strains of *Agaricus bisporus* (Lange) Sing under the agro-climatic conditions of Bihar

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Abstract

Mushroom cultivation is an eco-friendly, low-cost agricultural enterprise that utilises agro-industrial wastes to produce highly nutritious food while generating employment opportunities for rural communities. Among cultivated mushrooms, *Agaricus bisporus* (Lange) Sing (white button mushroom) is the most widely consumed species worldwide and accounts for a major share of global mushroom production. The present study was undertaken to evaluate the biological efficiency and morphological performance of six strains of *A. bisporus* (AVTB-24-201, AVTB-24-202, AVTB-24-203, AVTB-24-204, AVTB-24-205 and AVTB-24-206) under the agro-climatic conditions of Bihar. Parameters including biological efficiency, days to first harvest after casing, average fruit body weight and morphological traits were recorded and statistically analysed using a randomised block design (RBD). Among the tested strains, AVTB-24-201 exhibited the highest biological efficiency (19.02 kg/100 kg compost) and maximum average fruit body weight (26.07 g), indicating superior yield performance. The earliest harvesting was observed in AVTB-24-206 (17.04 days after casing), whereas AVTB-24-205 required the longest period for first harvest (26.88 days). Morphological evaluation revealed significant variation among strains, with AVTB-24-205 producing the longest stalk (3.03 cm), AVTB-24-204 exhibiting the greatest stalk width (8.36 mm) and cap length (16.82 mm) and AVTB-24-206 showing the maximum cap width (4.27 cm). The findings indicate considerable genetic variation among the evaluated strains, with AVTB-24-201 emerging as the most promising strain for commercial cultivation due to its superior yield and fruit body characteristics.

Keywords: *Agaricus bisporus*; biological efficiency; morphological characteristics; mushroom cultivation; spawn production; yield performance

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Introduction

Mushroom cultivation is a low-cost and sustainable enterprise, often described as “Kheti Bina Khet Ke,” that provides employment opportunities for rural communities while utilising agro-wastes and reducing environmental pollution. Mushrooms are rich in essential nutrients, including fatty acids (1, 2), proteins (3–5), dietary fibre (6), vitamins and minerals (7–9), polysaccharides (10) and polyphenols (11, 12). They lack cholesterol (13, 14) and offer health benefits such as blood pressure regulation (15) and anti-tumour properties (16). Unlike pulses and cereals, mushrooms contain essential amino acids like lysine, threonine, tryptophan, methionine and cystine, making them a valuable dietary supplement (17, 18). Mushrooms are often referred to as “superfoods” due to their rich content of selenium, vitamin D, glutathione and ergothioneine. The mushroom industry is categorised into edible mushrooms, medicinal mushroom-based products and wild mushrooms. Rising consumer demand for nutritious foods has increased interest in mushroom cultivation as a sustainable and efficient method of producing high-quality protein.

The white button mushroom, *Agaricus bisporus* (Lange) Sing, is an edible fungus classified under the phylum Basidiomycota. Owing to its widespread cultivation in over 70 countries, it has become one of the most popular and extensively consumed mushroom species globally. Indoor cultivation of *A. bisporus* began in caves in France during the late 18th to early 19th century (19). The cultivation of white button mushroom in India dates back to over 30 years ago, with Chambaghat, Solan recognised as one of the earliest centres of its commercial production. India offers significant opportunities for mushroom cultivation because of its

favourable agro-climatic conditions, plentiful agricultural by-products and affordable labour resources. White button mushroom (*A. bisporus*) farming is highly based on scientific and engineering activity involving four steps: composting, conditioning, spawning and casing (20). This mushroom is cultivated on compost, a specially prepared selective medium produced by bioconversion of agro-industrial, forestry and household organic/inorganic matters that favours growth of the mushrooms by practical exclusion of other microorganisms (21). Thus, the process of composting for button mushroom production is a solid-state fermentation process based on succession of thermophilic microbes and self-heating of substrate (22, 23).

Button mushroom (*A. bisporus*) dominates with a 70 % share (235200 tonnes), followed by oyster mushroom (*Pleurotus* spp.) at 17 % (57120 tonnes), paddy straw mushroom (*Volvariella volvacea*) at 9 % (30240 tonnes), milky mushroom (*Calocybe indica*) at 3 % (10080 tonnes) and other varieties, such as *Auricularia polytricha, Lentinus edodes* and *Flammulina velutipes,* at 1 % (3360 tonnes) (24). Mushroom cultivation has emerged as a profitable cottage industry for progressive farmers, offering supplementary income and promoting the productive recycling of farm wastes that were formerly disposed of or sold at minimal value. *Agaricus bisporus* is one of the most acceptable edible fungi contributing 31.8 % of total world mushroom production (25). Button mushroom is particularly suitable for temperate regions, with optimal cropping conditions of 15–25 °C temperature and 85–95 % relative humidity (RH). The global mushroom industry is largely concentrated in China, Russia and India, which collectively contribute more than 50 % of the total worldwide production. According to the National Horticulture Board, Bihar is the leading mushroom producer, contributing >28000 tonnes in 2021–22 (10.82 % of total production). Maharashtra (9.89 %) and Odisha (9.66 %) followed closely. Recent 2024 data from ICAR-Directorate of Mushroom Research, Solan shows Bihar leading with 41310 tonnes, followed by Odisha (32050 tonnes), Maharashtra (29290 tonnes) and Haryana (21950 tonnes).

Materials and Methods

The parent cultures of six *A. bisporus* strains, namely AVTB-24-201, AVTB-24-202, AVTB-24-203, AVTB-24-204, AVTB-24-205 and AVTB-24-206 were obtained from ICAR-Directorate of Mushroom Research, Solan under All India Coordinated Research Project on Mushroom at Advance Centre of Mushroom Research, Dr. Rajendra Prasad Central Agricultural University, Pusa Samastipur, Bihar.

Pure culture preparation

Pure cultures of each *A. bisporus* strain were maintained individually on potato dextrose agar (PDA) slants under aseptic conditions. The explants were surface sterilised using 0.1 % mercuric chloride ($HgCl_2$) and rinsed three times with sterile distilled water before inoculation. Cultures were incubated at incubation temperature 23–25 °C and periodically sub-cultured at monthly intervals to preserve their viability and fruiting characteristics.

Preparation of spawn

Healthy, disease- and insect-free wheat grains were selected and boiled with an equal volume of water until they became soft without rupturing. After boiling, the grains were spread under ambient conditions to remove excess surface moisture by air drying until the moisture content reached approximately 50–55 %. The partially dried grains were then amended with calcium carbonate (1 %, w/w) and calcium sulphate (2 %, w/w). The two additives were mixed thoroughly before being uniformly incorporated into the grains to adjust the pH and prevent grain clumping. The prepared substrate was transferred into 100 mL glass bottles and polypropylene bags (15 × 20 cm) and the openings were plugged with non-absorbent cotton. The containers were sterilised in an autoclave at 121 °C (15 psi) for 1 hr. Following sterilisation, they were allowed to cool for 24 hr before being inoculated aseptically with actively growing mycelial culture of *A. bisporus*. The inoculated containers were incubated at 23–25 °C for 23–28 days until complete mycelial colonisation of the grains was achieved. The fully colonised grain spawn was used immediately for mushroom cultivation.

Compost preparation

Since *A. bisporus* requires a selective composted substrate for optimal growth and fruiting, synthetic compost was prepared using the pipe method (short method of composting) following the protocol and formulation recommended by the Advanced Centre of Mushroom Research (ACMR), Dr. Rajendra Prasad Central Agricultural University (RPCAU), Pusa (Table 1).

Wheat straw was uniformly spread on a cemented platform and thoroughly moistened with water for 48 hr, with periodic turning to ensure uniform wetting. On the third day, the required quantities of urea, single superphosphate (SSP), muriate of potash (MOP), chicken manure or mustard cake, wheat bran and gypsum were separately moistened and thoroughly mixed with the pre-wetted straw.

On Day 0, the straw mixture was arranged into a heap approximately 7–7.5 ft wide, 1 ft high and 20 ft long. Perforated pipes were positioned within the heap to facilitate aeration, after which the heap was reconstructed and covered with black polyethylene. The composting process was continued for 15 days with periodic turning and temperature monitoring as described in Table 2. The prepared compost exhibited a C:N ratio of 16.4:1 and a pH of 7.6. The nitrogen content was 1.8 %, while the moisture content was 64 %. The

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compost was dark brown in colour, indicating proper decomposition and maturity.

Table 1. Pipe method of compost preparation

Components		Quantity
Wheat straw	:	1000 kg
Wheat bran	:	200 kg
Urea	:	20 kg
SSP	:	50 kg
MOP	:	10 kg
Chicken manure	:	300 kg
Mustard Cake	:	100 kg
Gypsum	:	25 kg
Plastic pipe	:	06

SSP: single superphosphate; MOP: muriate of potash.

Addition of spawn and filling of compost

After 15 days of composting, the compost was broken up completely and cooled to room temperature. Grain spawn was incorporated uniformly at 1.0 % (w/w) of the compost weight. The spawned compost was filled at 10 kg per polypropylene bag, with five replications maintained for each strain in a randomised block design (RBD). The bags were folded and incubated in a cropping room maintained at 22 ± 2 °C and 85–90 % relative humidity until complete spawn colonisation was achieved.

Casing

To stimulate fructification, a casing layer was applied after complete mycelial colonisation of the compost. The casing material consisted of an equal proportion (1:1, v/v) of well-decomposed farmyard manure and two-year-old spent mushroom compost. Following light compaction of the spawn-run compost, a uniform 4 cm thick casing layer was spread over its surface. After 4–5 days temperature of the mushroom growing room should be 15–19 °C. Mushrooms generally start appearing within 7–10 days. During this period, a humidity level of 85–90 % is required. Therefore, water should be sprayed as needed to maintain the required moisture.

Biological efficiency

The biological efficiency of *A. bisporus* was determined by recording the cumulative fresh yield obtained from successive harvests for each substrate across all 5 replicates. Biological efficiency (BE), which is the ability of mushrooms to convert the substrate contents into fruiting bodies was calculated by following (26):

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom yield}}{\text{Dry weight of substrate used}} \times 100 \quad \text{Eq. 1}$$

Morphological characteristics

The morphological characteristics of the six *A. bisporus* strains were recorded from fully developed fruiting bodies during the cropping period. Stalk length was measured from the base to the cap and stalk width at the widest portion using a scale. Cap width was measured across the widest portion, while cap length was measured along the longitudinal axis of the cap. All parameters were expressed in cm. The mean values were calculated from representative fruiting bodies collected from each replication.

Statistical methods

All experimental data were subjected to statistical analysis using the RBD through the latest version of the OPSTAT statistical software. The significance of differences among treatments was assessed by analysis of variance (ANOVA). Treatment means were compared using the critical difference (CD) test at the 5 % level of significance ($p \leq 0.05$).

Table 2. Composting schedule, temperature and operational conditions during preparation of *Agaricus bisporus* compost

Day	Composting operation
Day 0	Prepare the compost heap with perforated pipes and cover it with black polyethylene.
Days 1–5	Maintain the heap under cover for fermentation; temperature: 70–80 °C.
Day 6	Perform the first turning, reconstruct the heap and cover again; temperature: 65–70 °C.
Days 7–8	Maintain the heap under cover; temperature: 65–70 °C.
Days 9–11	Open the polyethylene from the sides to facilitate aeration.
Day 12	Perform the second turning, reconstruct the heap and cover from the top while keeping the sides open; temperature: 45–50 °C.
Day 14	Dismantle the heap and allow the compost to cool overnight.
Day 15	Carry out spawning after compost preparation; final compost pH: 7.2–7.8.

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Results and Discussion

Evaluation of the biological efficiency of *Agaricus bisporus* strains

The biological efficiency of six *A. bisporus* strains was assessed under identical cultivation conditions and the results are summarised in Table 3. Considerable differences in productivity were observed among the strains. AVTB-24-201 exhibited the highest biological efficiency, with a mean yield of 19.02 kg per 100 kg of compost, followed by AVTB-24-203 (17.46 kg per 100 kg). Intermediate yields were recorded for AVTB-24-204 (17.31 kg) and AVTB-24-206 (17.00 kg), whereas AVTB-24-205 (16.03 kg) and AVTB-24-202 (15.99 kg per 100 kg of compost) produced comparatively lower yields, with AVTB-24-202 performing the poorest among the tested strains. The minimum number of days to first harvest after casing was recorded in AVTB-24-206 (17.04 days), followed by AVTB-24-202 (18.44 days), whereas AVTB-24-205 required the maximum duration (26.88 days). The mean weight of individual fruiting bodies varied significantly among the six *A. bisporus* strains. AVTB-24-201 recorded the highest average fruit body weight (26.07 g), indicating superior fruit development. This was followed by AVTB-24-204 (22.88 g), AVTB-24-205 (22.77 g), AVTB-24-203 (21.88 g), AVTB-24-202 (21.04 g), whereas AVTB-24-206 produced the lightest fruiting bodies with an average weight of 20.19 g. The present findings are consistent with those of previous studies, which also reported significant variation in yield potential among different strains of *A. bisporus*. Among the evaluated strains, U-3 recorded the highest fruiting body count, producing 2161 mushrooms per 100 kg of compost (27). The next highest numbers were observed in strains S-649 and Pant-215. Similarly, another study observed substantial variability among *A. bisporus* strains in yield, fruiting body number, individual mushroom weight and other quality characteristics (28). Such variability is generally attributed to differences in the genetic potential of strains and their interaction with the cultivation environment.

Morphological characteristics of different *Agaricus bisporus* strains

The morphological characteristics of the six *A. bisporus* strains are presented in Fig. 1 and Table 4. Significant variation was observed among the strains for all the morphological traits evaluated. The longest stalk was recorded in strain AVTB-24-205 (3.03 cm), followed by AVTB-24-203 (2.84 cm), AVTB-24-206 (2.82 cm), AVTB-24-202 (2.47 cm) and AVTB-24-201 (2.27 cm), whereas the shortest stalk was observed in AVTB-24-204 (2.03 cm). The maximum stalk diameter was recorded in AVTB-24-204 (8.36 mm), followed by AVTB-24-205 (7.96 mm), AVTB-24-202 (7.47 mm), AVTB-24-206 (7.14 mm) and AVTB-24-203 (6.87 mm). The minimum stalk diameter was observed in AVTB-24-201 (6.84 mm). Cap diameter also differed significantly among the strains. AVTB-24-206 produced the widest caps (4.27 cm), followed by AVTB-24-205 (4.18 cm), AVTB-24-204 (3.95 cm), AVTB-24-203 (3.56 cm) and AVTB-24-202 (3.47 cm), while AVTB-24-201 recorded the smallest cap diameter (3.41 cm). Similarly, cap length exhibited considerable variation among the evaluated strains. The greatest cap length was observed in AVTB-24-204 (16.82 mm), followed by AVTB-24-205 (14.99 mm), AVTB-24-206 (13.16 mm), AVTB-24-202 (12.61 mm) and AVTB-24-203 (12.35 mm), whereas AVTB-24-201 recorded the shortest cap length (12.33 mm).

Table 3. Yield performance of different strains of *Agaricus bisporus*

Strain	Biological efficiency	First harvest days after casing	Average individual fruit body weight* (gm)
AVTB-24-201	19.02	21.44	26.07
AVTB-24-202	15.99	18.44	21.04
AVTB-24-203	17.46	23.06	21.88
AVTB-24-204	17.31	24.04	22.88
AVTB-24-205	16.03	26.88	22.77
AVTB-24-206	17.00	17.04	20.19
SEm	0.193	2.449	0.785
CD (5 %)	0.403	5.109	1.638

*Average of five replications.

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AVTB-24-201



AVTB-24-204



AVTB-24-203

Fig. 1. Morphological features of fruit body in six high yielding strains of *Agaricus bisporus*.

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Table 4. Morphological characteristics of different strains of *Agaricus bisporus*

*Strain	Length of stalk (cm)	Width of stalk (cm)	Width of mushroom cap (cm)	Length of mushroom cap (cm)
AVTB-24-201	2.27	6.84	3.41	12.33
AVTB-24-202	2.47	7.47	3.47	12.61
AVTB-24-203	2.84	6.87	3.56	12.35
AVTB-24-204	2.03	8.36	3.95	16.82
AVTB-24-205	3.03	7.96	4.18	14.99
AVTB-24-206	2.82	7.14	4.27	13.16
Sem	0.049	0.049	0.061	0.091
CD (5 %)	0.101	0.103	0.126	0.190

Based on the morphological observations, strain AVTB-24-205 exhibited the greatest average stalk length, whereas AVTB-24-204 recorded the maximum stalk width as well as the largest cap dimensions. In contrast, AVTB-24-201 generally showed the lowest values for most of the morphological traits studied. These findings demonstrate considerable variability among the evaluated *A. bisporus* strains with respect to fruiting body characteristics. The results obtained in the present investigation are in agreement with the observations of previous researchers, who reported significant cultural and morphological variation among seven strains of *A. bisporus* (CM-1, CM-5, CM-10, Delta, S-130, S-140 and X-13) and one strain of *A. bitorquis* (NCB-13) (29). Their study revealed differences in growth on malt extract agar, compost and casing soil, as well as variations in fruiting body characteristics. Strain S-130 exhibited superior growth on culture media, compost and casing soil, while NCB-13 produced the highest fruit body weight and stipe width. The shortest stipe length and largest pileus diameter were observed in strain Delta, whereas the maximum pileus thickness was recorded in CM-5. These observations support the existence of substantial strain-dependent variation in the morphological and yield-related attributes of cultivated mushrooms. Similarly, another researchers observed significant strain-to-strain variation in fruiting body morphology, yield and quality characteristics (28). Previous studies further demonstrated substantial genetic variability among *A. bisporus* strains and its association with differences in production performance (30). Differences in cap and stalk dimensions are important from a commercial perspective because fruiting body size, shape and appearance influence market acceptability. Therefore, the superior morphological attributes observed in AVTB-24-204, AVTB-24-205 and AVTB-24-206 indicate their potential for further evaluation under the prevailing production conditions. However, morphological characteristics should be considered together with yield, biological efficiency, earliness and quality traits for identifying the most promising strain. The present results are also supported by previous studies, which evaluated 8 strains of *A. bisporus* under different casing conditions (31). Significant differences were observed among strains in yield, number of fruiting bodies, pileus diameter, stipe length, colour and texture. The maximum pileus diameter was recorded in Delta and PB-5, whereas PB-5, U3 and S11 exhibited comparatively longer stipes.

Conclusion

The study revealed significant variation among the six strains of *Agaricus bisporus* in terms of biological efficiency and morphological traits. Strain AVTB-24-201 performed best, recording the highest biological efficiency and fruit body weight, while AVTB-24-206 showed the earliest harvesting. Morphological differences were also evident, with AVTB-24-205 producing the longest stalks and AVTB-24-204 exhibiting greater stalk width and cap size. Overall, AVTB-24-201 was identified as the most promising strain for commercial cultivation under the studied conditions due to its superior biological efficiency and higher fruit body weight. The adoption of high yielding strains such as AVTB-24-201 can improve farmers productivity and profitability. Further studies should focus on evaluating these strains under diverse agro-climatic conditions and optimising cultivation practices for sustainable mushroom production.

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Authors' contributions

RPP carried out the experiment, recorded observations and analysed the data. D formulated the research concept and guided the study. RPKR contributed to the study design, performed the statistical analysis and reviewed the manuscript. BP contributed to conducting the experiment and helped summarise and revise the manuscript. PK approved the final manuscript and helped summarise and revise it. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in limited portions of this manuscript solely for checking and improving grammatical accuracy and language clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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