2.3 Substrate preparation and bag inoculation

Clean, dry paddy straw sourced from local rice fields around Bansgadhi was chopped into 3-5 cm pieces and pasteurized using the hot-water immersion method (approximately 80 °C for one hour) in a drum heated over a wood-fired stove, after which the straw was drained and spread on a raised bamboo platform until it reached 65-70% moisture content, checked by the hand-squeeze test. Two kilograms of dry-weight-equivalent substrate was weighed for each bag on a digital balance and layered alternately with the assigned quantity of spawn inside perforated polypropylene bags (35 × 50 cm, 10 to 12 ventilation holes per bag, punched by hand rather than to a fixed template). Bags were sealed at the neck with cotton plugs and rubber bands, labelled by treatment and replication, and shifted to the incubation room within two hours of inoculation. Because hole number, diameter, and position were not standardized across bags, and additional cropping holes were later cut where colonization was uneven (Section 2.4), some bag-to-bag variation in ventilation and fruiting conditions cannot be ruled out; this is acknowledged as a limitation of the experimental setup (Section 4.4).

2.4 Crop management

Inoculated bags were incubated in the dark until mycelial colonization was visually complete (white mycelium fully covering the substrate), after which they were transferred to the fruiting room, the bag mouths opened, and cropping holes cut where colonization was uneven. Humidity was maintained through misting three to four times daily, and the room was ventilated by opening mesh-covered windows for 30 minutes each morning and evening to allow fresh-air exchange while excluding insects. No chemical fungicides or pesticides were applied at any stage; the only contamination-management measure was prompt removal and disposal of visibly infected bags to prevent spread to adjacent racks.

2.5 Observations and measurements

Days to complete spawn run, days to pinhead initiation, and days to first harvest were recorded from the date of inoculation for each bag. At every flush, the number of fruiting bodies, cap diameter, and stipe length were measured on five randomly tagged fruiting bodies per bag using a digital Vernier caliper (±0.01 mm) and averaged. Mushrooms were harvested at the button-to-early-flat stage across all flushes for 35 days from the date of transfer to the fruiting room, and total fresh yield per bag was recorded cumulatively using a digital balance (±0.1 g) and expressed on a g/bag and biological efficiency basis. Bags showing visible green (Trichoderma) or bacterial contamination before spawn run completion were recorded and excluded from subsequent yield measurement.

2.6 Biological efficiency and benefit-cost ratio

Biological efficiency (BE) was calculated as follows: BE (%) = (Total fresh mushroom yield ÷ Dry substrate weight) × 100. The benefit-cost ratio (BCR) for each treatment was estimated using a Bardiya farm-gate fresh mushroom price of NPR 250/kg and prevailing local input costs spawn at NPR 0.25/g and fixed non-spawn costs of NPR 40/bag (straw, bags, labour, and fuel for pasteurization) following BCR = Gross return ÷ Total cost of production.

2.7 Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) appropriate for a completely randomized design (CRD), following the procedures described by Gomez and Gomez (1984). Statistical analyses were performed using R software (version 4.3.1; R Core Team) through the RStudio interface. Prior to ANOVA, the assumptions of normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's tests, respectively. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at the 5% level of significance, and DMRT was used consistently as the sole method of mean comparison throughout Tables 1-3. Mean separation was performed using the agricolae package in R [AUTHOR TO CONFIRM: package version]. Microsoft Excel was used for data organization and summary calculations. For each parameter, the results are presented as treatment mean ± standard error (SE; n = 4), together with the ANOVA F-value, corresponding p-value, and DMRT letter groupings. The coefficient of variation (CV%) was calculated for each parameter.