rate does not appear to have been documented, and it may owe as much to spawn-packet sizing and grower habit as to deliberate optimization, though this has not been formally verified; comparable studies quantifying the growth, yield, and economic trade-offs of spawn rate under Nepalese Terai conditions remain scarce (Sapkota et al., 2022).

Most published spawn-rate work comes out of other South Asian contexts with different substrates, climates, and cost structures, which limits how directly it can be applied to Bardiya's growing conditions. On wheat straw in Pakistan, Bhatti et al. (2007) found that raising the grain-spawn rate progressively shortened the spawn run and that fresh-weight yield peaked at a 70 g/kg substrate rate (equivalent to about 7%, roughly 45% of substrate fresh weight), only marginally ahead of a 60 g/kg rate (about 6%, roughly 44%). Working with the related species Pleurotus pulmonarius on wheat straw in India, Pal et al. (2017) reported that increasing spawn rate from 0.5% to 8% shortened the spawn run to 10.5 days and pinhead formation to 12.3 days at 8%, which also gave the highest yield (168.7 g per 200 g dry substrate) and biological efficiency (84.3%), with only small gains in yield between 4% and 8%. Both studies point to a similar pattern of diminishing returns beyond a moderate spawn rate, broadly consistent with what was anticipated for Bardiya, but neither reported spawn cost or benefit-cost outcomes, and neither used paddy straw, underscoring the need for locally grounded data (Paudel and Dhakal, 2020).

This study was therefore undertaken to: (i) evaluate the effect of five spawn rates on mycelial colonization, pinhead initiation, and time to harvest; (ii) compare yield, yield-related morphological traits, and biological efficiency across spawn rates; and (iii) determine, through a simple benefit-cost analysis, the most economical spawn rate for smallholder oyster mushroom production in Bardiya, Nepal.

2 Materials and Methods

2.1 Experimental site

The experiment was conducted at Shree Janata Secondary School, Bansgadhi Municipality, Bardiya District, Lumbini Province, Nepal (28.26°N, 81.52°E; approximately 150 m above sea level), in a purpose-built mushroom cultivation shed adjoining the school science laboratory, from November 2024 to January 2025. The district has a subtropical monsoon climate with average annual rainfall of approximately 1,600-1,900 mm, most of it falling between June and September; during the trial period, ambient outdoor temperatures ranged from approximately 9 °C in December to 24 °C in November.

Because oyster mushroom production requires close environmental control, cultivation was carried out indoors rather than in the open field: the shed was constructed of brick walls with a corrugated tin roof lined with thatch for insulation, and incubation and fruiting activities were separated into two adjoining rooms. Incubation-room temperature was maintained at 24 °C-28 °C using ambient conditions supplemented by damp jute-sack curtaining on warmer afternoons, while the fruiting room was held at 18 °C-22 °C with 80%-90% relative humidity through regular hand-misting, and both rooms were monitored twice daily with a digital thermometer and hygrometer. The shed had not previously been used for mushroom cultivation, and all racks and surfaces were cleaned and disinfected with a 2% bleach solution before the experiment was established.

2.2 Experimental design and treatments

The experiment was laid out in a Completely Randomized Design (CRD) with five treatments and four replications, resulting in twenty experimental bags. The treatments were as follows: T_1: 4% spawn rate (80 g spawn/2 kg dry substrate); T_2: 6% spawn rate (120 g); T_3: 8% spawn rate (160 g); T_4: 10% spawn rate (200 g, farmer practice); T_5: 12% spawn rate (240 g).

Certified *Pleurotus ostreatus* grain spawn, obtained from a locally available commercial strain, was procured from Mushroom Seed Nepal & Research Center, Madhyapur Thimi-3, Chardobato, Bhaktapur, Nepal, a registered mushroom spawn producer, on November 5, 2025. The spawn was produced on wheat grain and used within one week of collection to preserve viability. Bags were arranged individually, rather than in field plots, on three-tier bamboo incubation racks, with treatments and replications randomized across rack positions to minimize any position-related microclimate effects within the shed.