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SHORT COMMUNICATION

EFFECT OF GENERATIONS OF CULTURE ON YIELD AND BIOLOGICAL EFFICIENCY OF *VOLVARIELLA VOLVACEA*

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Abstract: Successive sub-culturing of mushroom strains may lead to physiological degeneration and yield instability. The present investigation evaluated the effect of successive culture generations on spawn performance, yield attributes, and biological efficiency of *Volvariella volvacea* (paddy straw mushroom). Five culture generations (G0–G4), derived from a single high-performing isolate, were assessed under controlled cultivation conditions. Parameters studied included mycelial growth rate on PDA, spawn colonization time, primordia initiation, yield components, biological efficiency, and contamination incidence. Early generations (G0–G1) showed faster colonization, lower contamination, and significantly higher yield and biological efficiency. Later generations (G3–G4) exhibited delayed spawn run, increased variability in fruiting behavior, and symptoms of physiological degeneration. The results demonstrate progressive loss of culture vigor with repeated sub-culturing and highlight the importance of limiting serial transfers. Adoption of systematic strain preservation and rejuvenation practices is recommended to maintain consistent productivity in commercial cultivation of *V. volvacea*.

Keywords: *Volvariella volvacea*, Paddy straw mushroom, Culture generation, Spawn quality

INTRODUCTION

Volvariella volvacea, commonly known as the paddy straw mushroom, is an important tropical edible fungus widely cultivated in South and Southeast Asia. Productivity and economic viability of cultivation depend strongly on spawn quality. Repeated sub-culturing (serial transfers) of fungal strains can lead to genetic drift, physiological degeneration, or accumulation of contaminants, affecting yield and cultural characteristics. While degeneration and culture aging are well-documented in several cultivated mushrooms (e.g., *Pleurotus spp.*, *Agaricus bisporus*), systematic information for *V. volvacea* under standard spawn production practices is limited.

This study aims to quantify the influence of successive culture generations on spawn performance and crop yield and to provide practical recommendations for spawn handling in small-to-medium scale farms and institutional labs.

Objectives

1. To evaluate mycelial growth, spawn run and fruiting performance of *V. volvacea* across five successive generations (G0–G4) of culture.
2. To quantify changes in yield parameters (biological efficiency, average fruit weight, number of fruit bodies) and contamination rate across generations.
3. To make practical recommendations for spawn production and strain maintenance for growers.

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MATERIALS AND METHODS

Fungal material and isolation

A high-performing field isolate of *Volvariella volvacea* (labelled Vv-01) was obtained from a commercial grower near Bilaspur and purified by single-hyphal-tip transfer on potato dextrose agar (PDA). The founding culture (G0) served as the progenitor for generation series.

Generation scheme

Successive generations were produced by the following scheme:

- **G0** — founding, freshly purified single-hyphal-tip culture (master culture).
- **G1** — single transfer of G0 mycelium to fresh PDA (7–10 days incubation) then to grain spawns.
- **G2** — single transfer from G1 fruitbody tissue or from G1 PDA to fresh PDA, then to spawn substrate.
- **G3** — similarly transferred from G2.
- **G4** — transfered from G3.

Each generation used aseptic technique. All transfers followed identical incubation conditions (28±1°C; 70–80% RH) and were completed within 4 weeks per generation to standardize environmental effects.

Spawn production

Grain spawn used: sorghum grain soaked 12 h, boiled until al dente, drained, mixed with 2%

calcium carbonate, dispensed into 500 mL polypropylene bottles (200 g grain per bottle). Bottles were autoclaved at 121°C for 20 min and cooled. Each generation's inoculum consisted of 5 bottles of well-colonized PDA (or agar plugs) per 10 spawn bottles. Spawn was incubated at 28°C until full colonization.

Parameters recorded during spawn production:

- Time to 50% and 100% colonization (days)
- Contamination rate (%) per generation (number of bottles contaminated/total)

Substrate preparation and cropping

Substrate: chopped paddy straw (local variety) pasteurized by wet-heating in hot water at 60–65°C for 6 hours, drained to ~65% moisture, and filled into perforated polythene bags (5 kg wet straw per bag). Spawn rate: 2% (w/w) fresh straw basis. For each generation, 20 replicate bags were inoculated.

Cultivation conditions: incubation at 30±2°C in dark until spawn run (white mycelial cover), then low-light conditions (12 h photoperiod 500–1000 lux), humidity 85–90%, and temperature 30–33°C for fruiting (typical for tropical cultivation of *V. volvacea*). Pinning was induced by cold shock when applicable (brief exposure to 18–20°C) or by maintaining high humidity depending on local practice.

Data collection

For each replicate bag across generations, the following were recorded:

- Spawn run time (days to full colonization)
- Days to primordia initiation (from inoculation)
- Number of fruiting flushes observed within 30 days after primordia
- Total fresh weight of mushrooms per bag (g)
- Number of fruit bodies per bag
- Average fruit body weight (g) = total fresh weight / number of fruit bodies
- Biological efficiency (BE) = (total fresh weight of mushrooms / dry weight of substrate) × 100
- Incidence of abnormal fruiting or degeneration symptoms (thin stipes, small cap, delayed maturation)

Statistical analysis

Data were analyzed using one-way ANOVA with generation as the main factor. Post-hoc comparisons used Tukey's HSD. Contamination rates were analyzed using chi-square tests. Significance threshold set at $p < 0.05$. Where required, data were log-transformed to meet ANOVA assumptions.

RESULTS AND DISCUSSION

Spawn colonization and contamination

Culture generation significantly influenced spawn colonization rate and contamination incidence (Table 1). Early generations (G0–G1) colonized grain rapidly with minimal contamination, whereas later generations showed progressively slower colonization and higher contamination rates (χ^2, $p < 0.05$). Reduced competitive ability of physiologically aged mycelium may explain the increased contamination in later generations.

Fruiting behavior and degeneration

Later generations (G3–G4) exhibited delayed primordia initiation, uneven flushes, and increased frequency of malformed or aborted fruit bodies. Days to primordia initiation increased from 9.5 ± 1.2 days in G0 to 13.2 ± 2.1 days in G4, indicating progressive loss of physiological vigor.

Yield and biological efficiency

Yield and biological efficiency declined significantly with advancing culture generations (Table 2). G0 and G1 recorded the highest yields and BE, while G3 showed the lowest performance. Although G4 exhibited relatively higher mean BE, this was associated with high variability among replicates, indicating unstable performance rather than true recovery. Similar degeneration-related yield declines have been reported in other edible fungi.

Table 1. Effect of culture generation on spawn colonization and contamination rate in *Volvariella volvacea*

Culture generation	Time to 100% colonization (days)	Contamination (%)
G0	7.0 ± 0.7	2
G1	7.5 ± 0.8	3
G2	8.2 ± 0.9	5
G3	9.0 ± 1.1	8
G4	9.8 ± 1.3	12

Values are mean ± SD.

Table 2. Effect of culture generation on yield and biological efficiency of *Volvariellavolvacea*

Culture generation	Yield (g bag^{-1})	Fruit bodies (no. bag^{-1})	Avg. fruit wt (g)	BE (%)
G0	950 ± 85	42 ± 5	22.6	18.0 ± 3.4
G1	920 ± 95	40 ± 6	23.0	16.8 ± 4.1
G2	840 ± 110	36 ± 7	23.3	13.6 ± 4.8
G3	760 ± 140	33 ± 10	23.0	10.4 ± 6.0
G4	680 ± 160	29 ± 12	23.4	17.2 ± 7.2

Values are mean ± SD; differences significant at $p < 0.05$.

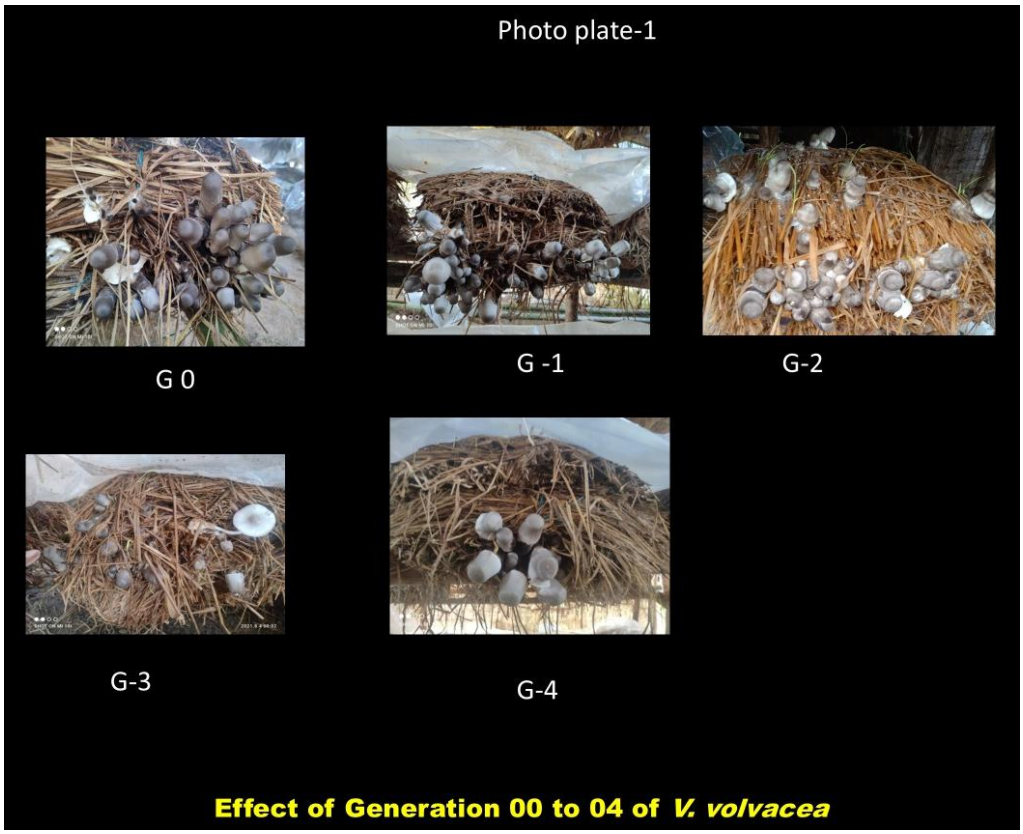

Fig.1. Effect of generation on yield in *Volvariella volvacea*

Repeated sub-culturing of fungal mycelia on artificial media is widely recognized as a major cause of strain degeneration in cultivated mushrooms. Degeneration manifests as reduced mycelialvigor, altered colony morphology, delayed primordia initiation, abnormal fruit bodies, and overall decline in yield potential (Chang and Buswell, 1996; Royse *et al.,* 2017). At the cellular level, degeneration has been linked to chromosomal instability, mitochondrial DNA rearrangements, epigenetic modifications, and reduced expression of lignocellulolytic enzymes essential for substrate colonization. In *Volvariella volvacea*, degeneration is often observed during routine spawn multiplication, particularly when cultures are repeatedly transferred without proper rejuvenation. Chang (1978) and Quimio (1982) emphasized that over-aged or repeatedly sub-cultured *V. volvacea* strains show erratic fruiting behavior, reduced fruit body size, and poor cropping uniformity. Oei (2005) further noted that spawn derived from aged cultures often exhibits delayed spawn run and increased contamination susceptibility. Evidence from other cultivated mushrooms supports the negative impact of serial sub-culturing. In *Pleurotus ostreatus*, repeated transfers resulted in slower substrate colonization, reduced enzyme activity, and lower biological efficiency (Sharma *et al.,* 2013). Similarly, degeneration in *Lentinula edodes* has been associated with delayed pinning, malformed fruit bodies, and reduced cropping cycles. In *Agaricus bisporus*, strain senescence has been linked to mitochondrial genome instability and reduced competitive ability during compost colonization. To mitigate degeneration, several strain preservation and rejuvenation techniques have been recommended, including periodic re-isolation from young and healthy fruit bodies, storage on agar slants under refrigeration, mineral oil preservation, sterile distilled water storage, and cryopreservation in liquid nitrogen. Stamets (2000) and Royse *et al.* (2017) emphasized that maintaining a master culture system and limiting the number of serial transfers are essential practices for sustaining long-term productivity in commercial mushroom cultivation. Despite these recommendations, quantitative studies evaluating the effect of defined successive culture generations on yield and biological efficiency of *V. volvacea* under standardized cultivation conditions are scarce. The present investigation addresses this gap by systematically assessing physiological performance, yield parameters, and degeneration symptoms across five successive generations (G0–G4) of pure culture.

CONCLUSION

Successive culture generations (G2–G4) of *Volvariella volvacea* produced through serial sub-

culturing showed measurable declines in spawn vigor, delayed fruiting, increased contamination, and reduced yield and biological efficiency compared to the founding culture (G0). Occasional higher values in later generations were inconsistent and associated with high variability. To sustain productivity, serial transfers should be minimized, master cultures maintained and periodic rejuvenation through fruit body re-isolation adopted.

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