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Production and Viability of White Liquid Culture Spawn of the Mushroom *Pleurocybella porrigens* on Crushed Maize Cobs

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Abstract

Pleurocybella porrigens (P. porrigens), an edible mushroom with high nutritional value, remains scarcely cultivated in Cameroon, primarily due to the lack of locally adapted spawn. Liquid spawn, though little studied in this context, represents a promising alternative to conventional solid spawn. This study evaluated the productivity of P. porrigens liquid spawn on different carbohydrate media (honey, glucose, fructose, sucrose), with or without iodized salt, and its ability to colonize crushed maize cobs. Mycelium was first isolated on PDA agar and subsequently inoculated into liquid media, incubated at 25–27 °C for 10 days. Five substrate treatments were tested, each with and without iodized salt. Parameters measured included Beginning of White Appearance (BWA), Major Substrate Colonization (MSC), and mycelial consistency at the first day (CWFD) and during major colonization (CMC), graded from + to ++++. Statistical analyses were performed using IBM SPSS Statistics (version 28), with three replicates per treatment (n = 3). The mycelium exhibited nearly linear growth on PDA agar at an average rate of 1.59 cm/day ($Y = 1.406X + 1.094$; $R^2 \approx 1$). In unsalted media, honey (A'3) and crushed cobs (A'5) promoted the earliest colonization (BWA: 2.00^b and 1.00^a; MSC: 2.00^b and 1.00^a), while other carbohydrate substrates (A'1, A'2, A'4) showed similar BWA (2.00^b) but slower MSC (3.00^c). In iodized media, honey (A3) maintained high efficiency (BWA = 3.00^a; MSC = 3.00^a), whereas glucose, fructose, and sucrose (A1, A2, A4) exhibited delayed colonization (BWA = 10.00^b; MSC = 13.00^c), and salted crushed cobs (A5) showed intermediate MSC (6.00^b). Differences in BWA and MSC were statistically significant ($p = 0.046$ and 0.017). The addition of iodized salt improved mycelial consistency in honey and glucose media ($p = 0.0214$), and a significant interaction between substrate type and salt affected MSC ($p = 0.0267$). Finally, honey-based liquid spawn successfully colonized crushed maize cobs within 96 hours, forming a dense, homogeneous mycelial mat (CMC = ++++). Variations in growth rate and pigmentation suggest that partial caramelization of honey during processing may influence mycelial development. These findings confirm the strong potential of honey-based liquid spawn as an effective, low-cost, and locally adaptable strategy for mushroom production in Cameroon.

Keywords: *Mycelial production, fungal spawn, liquid spawn, mycelial viability.*

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1. INTRODUCTION

Food security remains a major challenge in Cameroon, where population growth and economic constraints pressure national food systems. Edible mushrooms are a nutritive and economically viable resource, providing proteins, essential amino acids, vitamins, and bioactive compounds, contributing to household nutrition and rural livelihoods (Royse et al., 2020). Cultivation requires low capital investment and can be integrated into smallholder farming, promoting food security and income diversification (Royse et al., 2020; Rashid et al., 2021).

Globally, mushroom production has increased over recent decades, driven by dietary diversification and demand for sustainable foods (Singh et al., 2022). In Africa, rising consumption strengthens their role in improving nutrition and alleviating poverty (Rashid et al., 2021). However, technological limitations in spawn production restrict mushroom cultivation in many Sub-Saharan countries, including Cameroon (Kumla et al., 2020).

Conventional cultivation relies on solid spawn, prone to contamination, inconsistent quality, and limited shelf life (Kumla et al., 2020). Liquid spawn offers faster mycelial growth, uniform biomass, better sterility control, and scalability (Zied et al., 2022) yet remains insufficiently explored for *P. porrigens* under Central African conditions.

P. porrigens, a Basidiomycota in Agaricales (Marasmiaceae), is saprotrophic, growing on decayed wood with small to medium white fruiting bodies (Watanabe et al., 2024). Genomic and transcriptomic analyses reveal rapid mycelial growth and enzymatic potential suitable for liquid cultivation (Watanabe et al., 2026; Adachi et al., 2025).

Mycelial growth in liquid media is influenced by carbon sources and mineral balance. Glucose, fructose, and sucrose affect biomass and growth kinetics in edible mushrooms (Maseko et al., 2024; Wang et al., 2025), while excessive mineral salts may inhibit growth (Mleczek et al., 2020). Agricultural residues, such as crushed maize cobs, are promising for spawn survival and colonisation (Kortei et al., 2020).

Despite studies on liquid spawn in other species, knowledge is limited for *P. porrigens* regarding nutrient media optimisation, mineral supplementation, and substrate colonisation. This gap justifies the present study. The general objective was to evaluate the productivity of liquid spawn in different sweet liquid media (honey, glucose, fructose, and sucrose) with or without iodised salt and to assess the viability of the best spawn on crushed maize cobs, specifically characterising mycelial development during pure strain isolation, comparing nutrient solutions with and without iodised salt, and evaluating viability and colonisation of the produced spawn.

2. MATERIALS AND METHODS

2.1. Study Area and Sites

The study was conducted in Ebolowa, southern Cameroon, and a region with a warm and humid equatorial climate (22–30 °C) favourable for mushroom cultivation. Two complementary locations were used:

- **Laboratory-based seed production and quality control** at the Regional Hospital of Ebolowa.

- **Cultivation experiments** in a semi-controlled mushroom house established in a disinfected former retail space.

A wooden shelf system, thoroughly cleaned and elevated from the floor, was installed to simulate realistic cultivation conditions while allowing rigorous monitoring of liquid spawn viability and mycelial growth.

2.2. Biological Material

The experimental substrates included D-glucose, D-fructose, and D-sucrose, purchased in Bafoussam; honey and potatoes, sourced from the local market in Ebolowa; and Sabouraud agar supplemented with chloramphenicol, as well as distilled water, provided by the laboratory. Lime, acquired locally, was used for sterilising PET containers and disinfecting the mushroom house. Crushed maize cobs served as the control substrate for evaluating spawn viability. The solid spawn of *P. porrigens* was obtained from a regional mushroom producer.

2.3. Experimental Methods

2.3.1. Characterisation of *P. porrigens* Mycelium during Incubation

2.3.1.1. Preparation of PDA culture medium

All non-biological materials were sterilised prior to use. The Potato Dextrose Agar (PDA) medium was prepared according to the protocol described by Bedidjo et al. (2024). Briefly, 200 g of potatoes were boiled in 150 mL of distilled water for 20–25 minutes to obtain a filtered potato broth. Separately, 30 g of agar-agar was dissolved in 150 mL of hot distilled water. The two solutions were then combined, and 15 g of sucrose was added. The final volume was adjusted to 350 mL with distilled water, and the medium was sterilised at 125 °C for 15 minutes. After sterilisation, the medium was cooled under a laminar flow hood, poured into sterile Petri dishes, and allowed to solidify at 4–5 °C for 24–48 hours.

2.3.1.2. Inoculation and incubation on PDA medium

Under a laminar flow hood, a small fragment (<1 cm) of solid *P. porrigens* spawn was aseptically excised using a sterile scalpel and inoculated at the centre of the Petri dishes containing solidified PDA medium (Traoré et al., 2025). The dishes were sealed and wrapped with plastic film, then incubated at 27 °C for 7 days, with the inoculated surface facing downward (Traoré et al., 2025). Mycelial growth was measured daily in centimetres (Maseko et al., 2024; Wang et al., 2025). Morphological characteristics of the mycelium were observed and recorded according to the criteria established by Mimouni (2020).

Observations conducted on PDA media enabled a macroscopic assessment of the appearance, colour, and structure of *P. porrigens* mycelium during incubation. Measurement of mycelial diameter (cm) allowed monitoring of growth and graphical representation of its progression over time, thereby characterising the dynamics of development, particularly the onset of active growth and the increase in colony diameter.

2.3.2. Comparison of Nutrient Solutions for Liquid Seed Production

2.3.2.1. *Preparation of nutrient solutions:*

Four glass jars (A1–A4), each fitted with perforated lids containing cotton and polyester filters, were filled with 185 mL of distilled water supplemented with 10 g of a carbohydrate source (glucose, fructose, honey, or sucrose) and 5 g of mineral salts. Each formulation was prepared in triplicate (n = 3). The jars were sterilised at 121 °C for 20 minutes, then allowed to cool and stored under aseptic conditions. A second set of jars without mineral salts (A'1–A'4), also prepared in triplicate, and followed the same procedure. The detailed composition of the nutrient formulations is presented in Table 1.

Table 1: *composition of nutrient media used for mycelial culture*

Jar	Carbohydrate Source	Quantity (g)	Mineral Salts (g)	Volume (ml)
A1	Glucose	10	5	185
A2	Fructose	10	5	185
A3	Honey	10	5	185
A4	Sucrose	10	5	185

A1: Salted glucose nutrient solution; A2: Salted fructose nutrient solution; A3: Salted honey nutrient solution; A4: Salted sucrose nutrient solution.

2.3.2.2. Preparation of control solid substrates based on maize cob powder

Maize cob powders were prepared following the method described by Mimouni (2020) and placed in jars fitted with perforated lids for sterilisation. Two control substrates were established: A5 (with mineral salts) and A'5 (without mineral salts), each prepared in triplicate. These controls were used to compare the viability of spawn derived from solid and liquid media while accounting for differences related to aeration, moisture content, and substrate structure under controlled conditions.

2.3.2.3. Preparation of Liquid and Solid Seeds of *P. porrigens*

The preparation of liquid and solid seeds was conducted in three successive phases under standardised conditions:

- ***Initial phase:***

Pure mycelial fragments (~1 cm) from PDA plates were inoculated into liquid media (A1–A4, A'1–A'4) and solid media (A5, A'5), each in triplicate, and incubated at 25–27°C for 15 days. Iodised salt (5 g) was added only to A1–A4. Evaluated parameters included onset of Beginning Mycelium Appearance (BMA) and Major Substrate Colonisation (MSC), using a qualitative ranking system. Substrate A3 (honey + salt) exhibited the fastest and most extensive mycelial growth.

- ***Secondary phase and microscopic observation:***

Daughter cultures T3 and T'3 (n = 2) were generated from liquid spawn in A3 and A'3. A dark pigmentation appeared in A'3 and T'3, likely due to commercially purchased honey, which may have undergone partial caramelisation during processing or transport, affecting its natural composition. Microscopic analysis (×400) confirmed intact and normal mycelial morphology, indicating that the pigmentation did not compromise growth or structural integrity. This highlights the importance of honey quality for liquid spawn performance.

- ***Final phase:***

Daughter liquid spawn (T3 and T'3) was inoculated into sterilised and decanted honey under four treatments: T3 salted, T3 unsalted, T'3 salted, and T'3 unsalted (n = 2 per treatment). Cultures were gently and regularly agitated to prevent mycelial clumping and ensure uniform growth. Subsequent colonisation on crushed maize cobs was assessed using a semi-quantitative visual scale, where mycelial density was coded numerically as follows: 1 = low (+), 2 = moderate (++), 3 = high (+++), and 4 = very dense and homogeneous (++++). This approach allowed a more reproducible and objective comparison of spawn performance across treatments while maintaining qualitative observations of mycelial texture and consistency.

This methodology allowed for the comparison of different nutrient solutions for liquid spawn production by evaluating, both qualitatively and semi-quantitatively, the Beginning Mycelium Appearance (BMA), defined as the initial visible emergence of white mycelium, and Major Substrate Colonisation (MSC), representing extensive mycelial coverage of the substrate. These assessments provided insight into the earliness and overall extent of mycelial development across substrates. Additionally, subsequent inoculation of crushed maize cobs enabled the evaluation of the practical performance and viability of the liquid spawn, with solid spawn used as a control to benchmark colonisation speed.

2.3.3. Evaluation of spawn viability on crushed maize cobs

2.3.3.1. Substrate sterilisation and inoculation

Crushed maize cobs were washed, soaked, and sterilised at 100 °C for 7 hours. Each PET bottle (n = 2 per treatment) with perforated lids (eight 1-cm holes cut three-quarters around the circumference) was filled with boiled and moistened maize cobs.

Substrates were inoculated either with 10 mL of liquid spawn (T3/T'3 salted/unsalted) injected centrally or with 30 g of solid spawn (A5) distributed in perforations and the centre. Bottles were sealed, plugged with sterile cotton, and incubated at room temperature in darkness with adequate aeration (Mimouni, 2020).

Table 2: *Treatments and Number of Inoculated PET Bottles*

Treatment Type	Bottle Name	Number of Bottles	Description
*T3 unsalted	PET-*T3-unsalted	2	Injection of 10 ml, sealed with sterile cotton
*T3 salted	PET-*T3-salted	2	Same as above
*T'3 unsalted	PET-*T'3-unsalted	2	Same as above
*T'3 salted	PET-*T'3-salted	2	Same as above
A5 (solid)	PET-S	2	30 g placed in holes + a pinch in center

**T3 unsalted/salted = seeds from T3 inoculated in sterilized and decanted honey; *T'3 unsalted/salted = seeds from T'3 inoculated in sterilized and decanted honey; PET-*T3/T'3 = corresponding fruiting bodies; PET-S = fruiting bodies from solid corn cob seed (A5).*

2.3.3.2. Evaluation of Liquid Spawn Efficiency

The efficiency of liquid spawn was assessed on PET substrates prepared from crushed maize cobs. Mycelial development was monitored over a 5-day period and evaluated using a semi-quantitative macroscopic scale, coded numerically as follows: 0 = absent, 1 = slightly visible, 2 = moderately visible, and 3 = abundant.

In addition to density, mycelial colour and texture were systematically recorded using standardised visual assessment criteria, allowing consistent comparison of growth patterns across treatments. This combined approach ensured both qualitative and semi-quantitative evaluation of spawn performance, providing robust data on colonisation efficiency.

3. STATISTICAL ANALYSES

Data analyses were conducted using Microsoft Excel for graphical representation, calculation of means, standard deviations, and model fitting (R^2). Comparisons of spawn performance, specifically the onset of Beginning Mycelium Appearance (BMA) and Major Substrate Colonisation (MSC), were performed using the non-parametric Kruskal–Wallis test in IBM SPSS Statistics (version 25), with the significance level set at 5% ($p < 0.05$).

This statistical approach was chosen due to the non-normal distribution of the data, the heterogeneity of variances, and the near-absence of intra-group variability, as reflected by almost zero standard deviations (n = 3 per treatment). Under these circumstances, the raw data were retained in their original form and primarily interpreted through graphical representation, which enabled clearer visual comparisons among substrates.

When significant differences were observed, Dunn's post hoc test with Bonferroni correction was applied to determine statistically distinct groups, ensuring a rigorous and reproducible evaluation of spawn performance across treatments.

4. RESULTS AND DISCUSSION

4.1. Characterisation of *P. porrigens* Mycelium after Incubation

This section reports the macroscopic characteristics of *P. porrigens* mycelium derived from pure strain cultures.

4.1.1. Visual Appearance on Petri Dishes

Figure 1 shows the mycelial growth of *P. porrigens* on Potato Dextrose Agar (PDA) plates after 5 days of incubation at 37°C. The mycelium displayed a dense, whitish, and cottony texture, uniformly covering the medium. Petri dishes labelled P1 to P6, inoculated with solid *Pleurocybella porrigens* spawn and incubated for 7 days, demonstrated extensive colonisation across the surface.

Figure 1: *Macroscopic appearance of Pleurocybella porrigens mycelium on PDA plates after 7 days at 37 °C (P1–P6).*

4.1.2. Mycelial growth rate on PDA medium

The progression of *P. porrigens* mycelium diameter (cm) over a 5-day incubation period on PDA and Sabouraud chloramphenicol agar media, with six replicates (n = 6), showed an initial increase of approximately 2.5 cm between day 0 and day 1, corresponding to a lag phase during which the mycelium adapted to the culture environment, resulting in limited and irregular growth. From day 1 to day 5, growth became linear and steady, with an average rate of 1.59 cm per day. This pattern fits the linear regression model Y = 1.406X + 1.094 (Y = diameter in cm; X = time in days), with a coefficient of determination $R^2 = 0.9877$, indicating an excellent fit to the data (Figure 2).

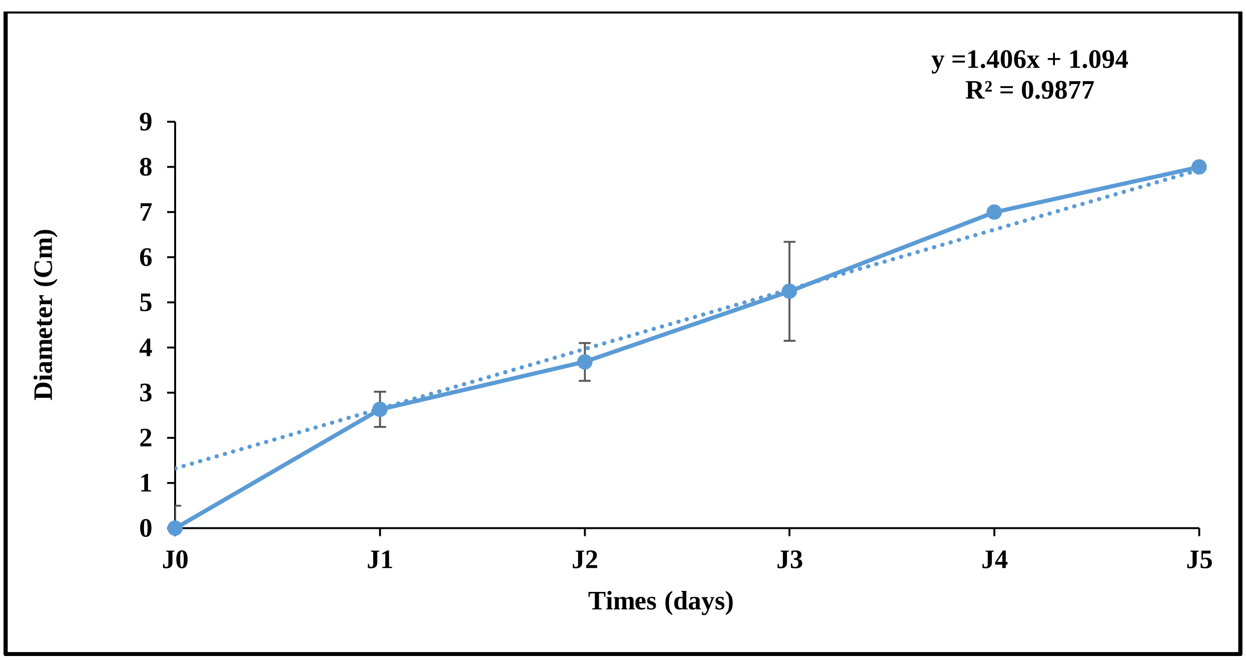

Figure 2: *Mycelial growth rate of P. porrigens on PDA medium over 5 days.*

4. 2. Comparison of Nutrient Solutions to produce *P. porrigens* Spawn in White Liquid Cultures.

4.2.1. Appearance of *P. porrigens* White Mycelium across Nutrient Solutions After Incubation

Macroscopic observations of the incubated spawn are shown in Figure 3, illustrating the appearance and colonization of *P. porrigens* white mycelium on various liquid and solid substrates enriched with iodized salt. These images allow direct comparison of mycelial consistency in terms of density, homogeneity, and texture after incubation. After 15 days, the liquid media containing glucose (A1), fructose (A2), and sucrose (A4) exhibited minimal turbidity, with only a faint cloudy film visible. In contrast, the honey-based medium (A3) displayed a noticeable lightening of color, reflecting more active mycelial growth. On the solid substrate (A5), *P. porrigens* white mycelium completely colonized the crushed maize cobs, demonstrating a high capacity for substrate (Figure 3).

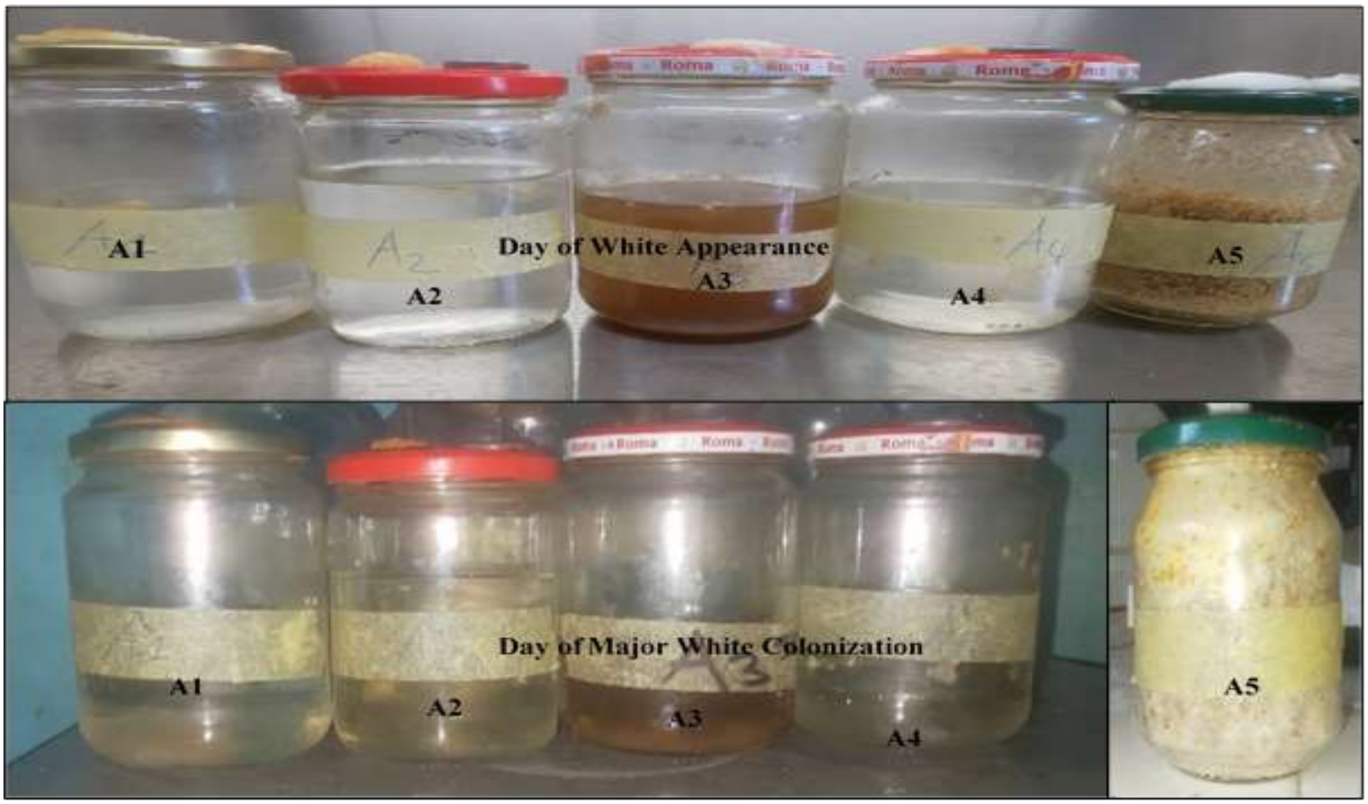

Figure 3: *Growth of different P. porrigens inocula (liquid and solid spawn) with iodized salt after 15 days of incubation (n = 3).*

After 15 days of incubation, the liquid media without iodized salt generally showed reduced mycelial development compared to their salted counterparts. The honey-based medium without salt (A’3) still supported some mycelial growth, as evidenced by slight turbidity, whereas the glucose (A’1), fructose (A’2), and sucrose (A’4) media remained mostly clear, with minimal visible mycelium. In contrast, the solid corn cob substrate without salt (A’5) exhibited more uniform and denser mycelial colonization than the liquid media, confirming its suitability as a solid growth support even in the absence of added salts (Figure 4).

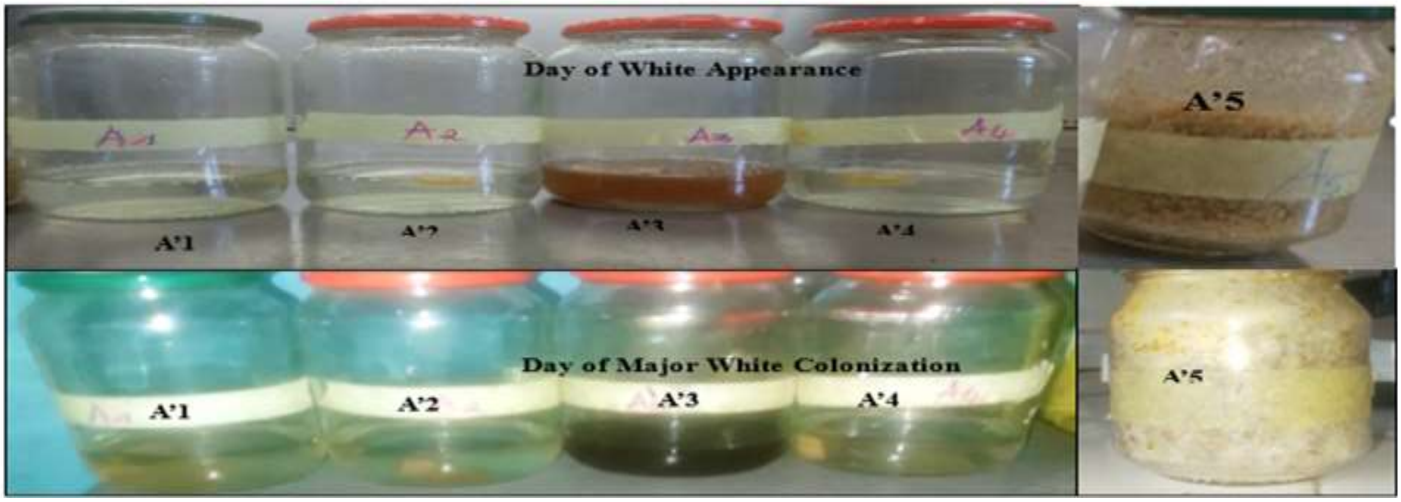

Figure 4: *Appearance and colonization of P. porrigens white liquid spawn after 15 days of incubation without iodized salt (n = 3).*

4.2.2. Rate of mycelium emergence

The comparison of mean ranks for two parameters, Beginning of White Appearance (BWA) and Major Substrate Colonization (MSC), on liquid substrates supplemented with iodized salt revealed significant differences between formulations. The honey-based substrate (A3) exhibited a low mean rank for BWA (3[a]), indicating an early onset of mycelial growth, whereas substrates A1 (glucose), A2 (fructose), A4 (sucrose), and A5 (maize cobs) showed higher values (10[b]), reflecting delayed initiation of growth, with no significant differences among them. Regarding MSC, A3 again demonstrated the fastest colonization (3[a]), followed by A5 (6[b]), which significantly outperformed the simple carbohydrate substrates (A1, A2, A4) that had the highest mean ranks (13[c]), indicating slower substrate colonization.

These results highlight the superior efficiency of honey as a liquid substrate, likely due to its biochemical complexity, including simple sugars, enzymes, organic acids, and trace elements that promote fungal growth. Although lignocellulosic, substrate A5 also showed good colonization performance, confirming its relevance as a solid support even in the presence of iodized salt. In contrast, simple carbohydrate substrates, despite their high energy content, appeared less stimulatory, possibly due to limited nutritional diversity or unfavorable interactions with the added salts (Figure 5).

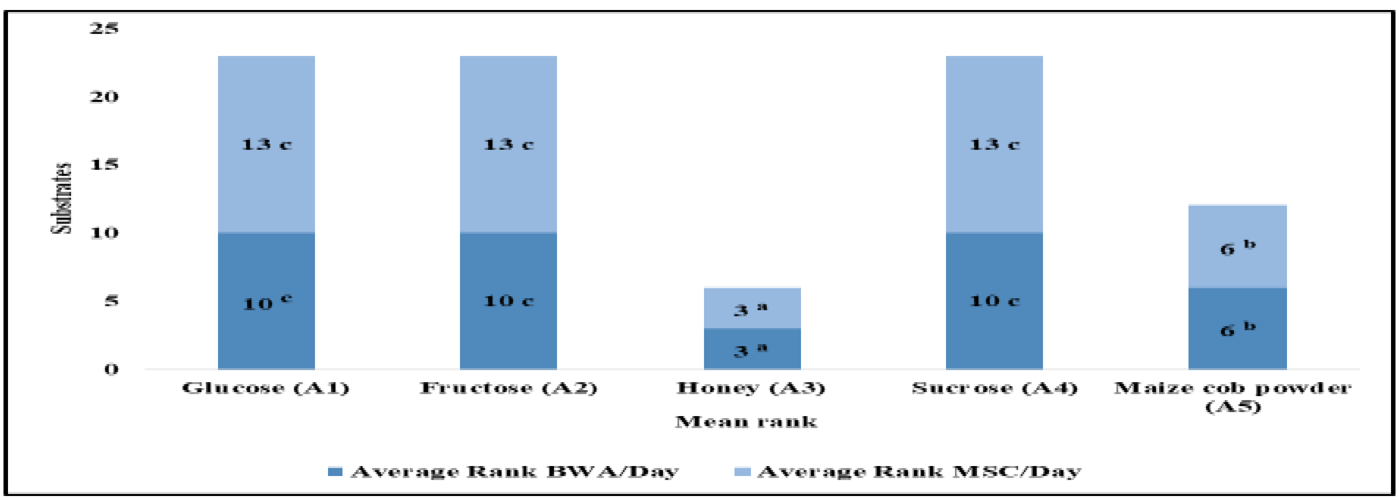

Figure 5: *Average ranks of Beginning of White Appearance (BWA) and Major Substrate Colonization (MSC) on liquid substrates with iodized salts (n = 3).*

Substrates: A1 = water + glucose, A2 = water + fructose, A3 = water + honey, A4 = water + sucrose, A5 = maize cob powder, all with mineral salts. Superscript letters ([a], [b], [c]) indicate statistically different groups (Dunn's post hoc test, Bonferroni correction, $p < 0.05$).

The analysis of mean ranks for the Beginning of White Appearance (BWA) showed no significant differences among the carbohydrate-based substrates A'1 (glucose), A'2 (fructose), A'3 (honey), and A'4 (sucrose), all exhibiting an identical mean rank of 2.00[b]. This indicates a comparable rate of mycelial initiation across these media, despite minor variations in actual onset times. In contrast, substrate A'5 (non-salted crushed maize cobs) displayed a significantly lower rank (1.00[a]), reflecting earlier mycelial emergence.

Regarding Major Substrate Colonization (MSC), substrates A'1, A'2, and A'4 had the highest mean rank (3.00[c]), indicating slower colonization. The honey-based medium (A'3) showed intermediate performance (2.00[b]), while A'5 again demonstrated the fastest colonization with the lowest mean rank (1.00[a]).

These results highlight that, even in the absence of iodized salt, substrate composition strongly affects the growth dynamics of *P. porrigens*. Crushed maize cobs (A'5) were particularly favorable for rapid mycelial development, while the honey-based medium (A'3), rich in bioactive compounds, also supported efficient growth. By contrast, simple sugar media, despite their high energy content, were less effective in sustaining extensive mycelial colonization (Figure 6).

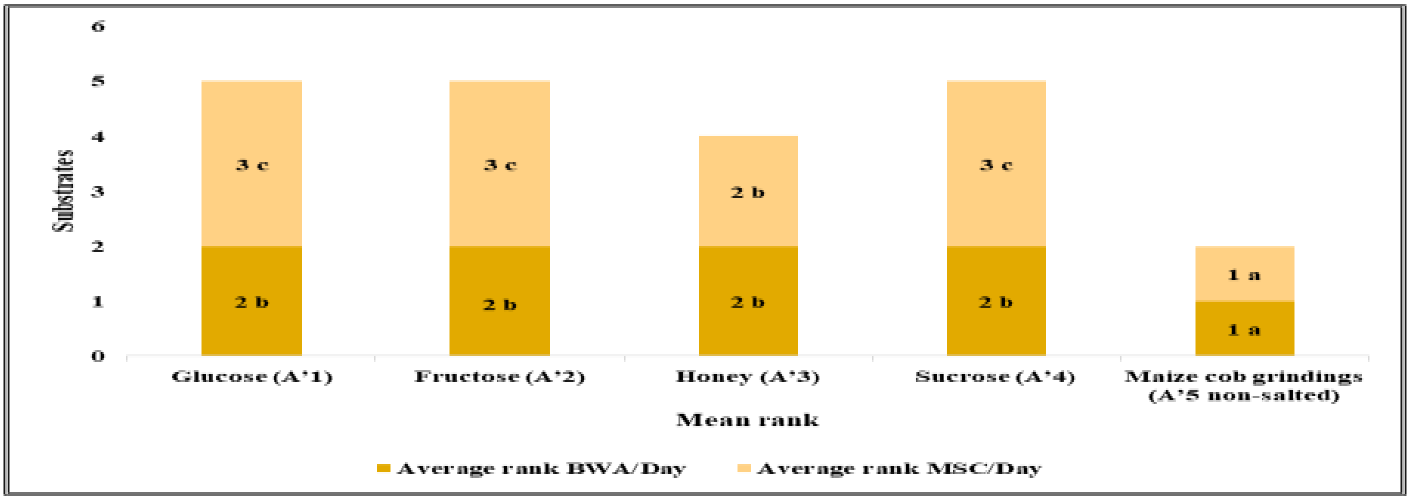

Figure 6: *Average ranks of Beginning of White Appearance (BWA) and Major Substrate Colonization (MSC) on liquid substrates with non-salted (n = 3).*

Substrates: A1 = water + glucose, A2 = water + fructose, A3 = water + honey, A4 = water + sucrose, A5 = maize cob powder, all with mineral salts. Superscript letters ([a], [b], [c]) indicate statistically different groups (Dunn's post hoc test, Bonferroni correction, $p < 0.05$).

4.2.3. Consistency of *P. porrigens* white mycelium

After 15 days of incubation, the white mycelial consistency of *P. porrigens* was evaluated in different liquid substrates supplemented with iodised salt. Consistency was assessed based on density, homogeneity, and texture at two stages: the first visible appearance of white mycelium (CWAFD) and the stage of major colonisation (CMWC). The results indicate that the honey-based medium (A3) exhibited the highest initial consistency (++++), followed by glucose (A1, +++). Fructose (A2) and corn cob grindings (A5) showed moderate initial consistency (++), whereas sucrose (A4) recorded the lowest score (+). At the stage of major colonisation, corn cob grindings (A5) demonstrated the highest consistency (++++), followed by honey (A3, +++). Glucose (A1), fructose (A2), and sucrose (A4) exhibited comparable moderate consistencies (++) (Table 3).

Table 3: *white mycelium appearance of P. porrigens in liquid substrates enriched with iodized salt.*

Different substrates for seed production	Consistency of White Appearing on First Day (CWAFD)	Consistency of Major White Colonization (CMWC)
Glucose (A1)	+ + +	+ +
Fructose (A2)	+ +	+ +
Honey (A3)	+ + + +	+ + +
Sucrose (A4)	+	+ +
Corn cob grindings (A5)	+ +	+ + + +

A1: distilled water + glucose + mineral salts; A2: distilled water + fructose + mineral salts; A3: distilled water + honey + mineral salts; A4: distilled water + sucrose + mineral salts; A5: corn cob grindings + mineral salts. Mycelial consistency was classified as very low (+), low (++), medium (+++), and high (++++). CWAFD = Consistency of White Appearance on First Day; CMWC = Consistency of Major White Colonisation (n = 3).

Table 4 presents the white mycelial consistency of P. porrigens after 15 days of incubation in liquid substrates prepared without iodised salt. As in the previous experiment, consistency was evaluated at the first day of visible white appearance (WCAFD) and at the stage of major colonisation (CMWC).

The honey-based substrate (A'3) exhibited high consistency at both stages (++++). Corn cob powder (A'5) showed moderate initial appearance (++) but high consistency at major colonisation (++++). Glucose (A'1) and fructose (A'2) displayed moderate to high consistencies (+++), whereas sucrose (A'4) recorded comparatively lower values, particularly at the major colonisation stage (+).

Table 4: *White mycelial consistency of P. porrigens in liquid substrates without iodised salt.*

Different substrates for seed production	White Consistencies Appeared on the First Day (WCAFD)	Consistencies of Major White Colonization (CMWC)
Glucose (A'1)	+ + +	+ + +
Fructose (A'2)	+ + +	+ + +
Honey (A'3)	+ + + +	+ + + +
Sucrose (A'4)	+ +	+ +
Corn cob powder (A'5)	+ +	+ + + +

A'1: distilled water + glucose; A'2: distilled water + fructose; A'3: distilled water + honey; A'4: distilled water + sucrose. The consistency of Pleurocybella p. mycelium is classified into four categories: very low (+), low (++), medium (+++), and high (++++). WCAFD refers to White Consistencies Appeared on the First Day, and CMWC to Consistencies of Major White Colonization (n = 3).

4.2.4. Microscopic observation and assessment of mycelial filaments

Microscopic examination was conducted on mycelial filaments obtained from liquid white spawn cultures T3 and T'3 (n = 2), derived respectively from the enriched honey-based substrate (A3, supplemented with iodised salt) and the non-enriched honey substrate (A'3, without iodised salt). Both cultures exhibited dark pigmentation at the macroscopic level. However, microscopic analysis revealed clearly defined and structurally organised filamentous networks in both samples. The mycelial filaments observed in T3 appeared denser and more compact, with greater interconnectivity compared to those in T'3, which showed a comparatively looser arrangement. These observations indicate a difference in filament density between the iodised salt-supplemented and non-supplemented honey-based liquid media (Figure 4).

Figure 7: *Microscopic observations of mycelial filaments from T3 (honey-based liquid spawn with iodised salt) and T'3 (honey-based liquid spawn without iodised salt).*

4.2.5. Macroscopic Observation of Final Honey-Based Liquid Spawn Preparations

The final liquid spawn preparations obtained after 15 days of incubation are presented in Figure 5. These include T3 non-salted and T3 salted, both derived from the T3 culture, as well as T'3 non-salted and T'3 salted, derived from the T'3 culture (n = 2). All preparations were produced from honey-based liquid substrates.

Macroscopic observation shows that both T3 and T'3 liquid spawn exhibited dark pigmentation, consistent with the appearance previously observed in the parent seed. Despite this visible colour change, the cultures maintained homogeneous dispersion of mycelial biomass within the liquid medium. Differences in visual density were noted between treatments, with salted preparations appearing more compact compared to their non-salted counterparts. These observations indicate variations in biomass distribution between iodised salt-supplemented and non-supplemented liquid spawn preparations.

Figure 8: *Macroscopic appearance of final honey-based liquid spawn preparations: T3 non-salted, T3 salted, T'3 non-salted, and T'3 salted (n = 2).*

4.3. Evaluation of the Viability of Seeds Produced in White Liquid Cultures of *P. porrigens* on Corn Cob Grindings

4.3.1. Colour and Texture Characteristics of the Mycelium

The macroscopic colour and texture characteristics of *P. porrigens* mycelium were assessed on fertile PET substrates five days after inoculation. The evaluated treatments included salted PET-solid (4), PETT'3 non-salted (1), PETT'3 salted (2), PETT3 non-salted (3), and PETT3 salted (5) (n = 2), as presented in Figure 6.
macroscopic features. Irrespective of the type of inoculum

After five days of incubation, all fruiting substrates contained in PET bottles showed comparable used (liquid or solid; salted or non-salted), the mycelium displayed a characteristic cottony texture. Colouration ranged from pure white to pale yellow-orange across treatments. No marked structural abnormalities were observed. The overall uniformity in texture and colour among treatments indicates consistent mycelial establishment on the corn cob grinding substrates under the tested conditions.

Figure 9: *Macroscopic appearance of P. porrigens fruiting substrates five days after inoculation. 4: salted PET-solid; 1: PETT'3 non-salted; 2: PETT'3 salted; 3: PETT3 non-salted; 5: PETT3 salted (n = 2).*

4.3.2. Assessment of the Onset of *P. porrigens* Mycelial Appearance

The temporal progression of *P. porrigens* mycelial growth on PET-based fruiting substrates was monitored over 120 hours and is presented in Figure 8. Distinct growth patterns were observed depending on the type of inoculum used. The PET-solid substrate inoculated with solid spawn (A5) showed delayed mycelial establishment, characterised by a lag phase extending up to 48 hours. Subsequent growth progressed gradually, reaching a score of 3 units at 120 hours. In contrast, PET-T3-salted, PET-T3-unsalted, PET-T'3-salted, and PET-T'3-unsalted substrates, all inoculated with liquid spawn, exhibited similar growth kinetics. Visible mycelial development was recorded at 48 hours, followed by steady expansion up to 72 hours. Maximum recorded growth (3 units) was reached at 96 hours, after which growth stabilised. Overall, liquid spawn treatments demonstrated earlier onset of visible colonisation and faster attainment of maximum growth scores compared to the solid spawn treatment under the same experimental conditions (Figure 8).

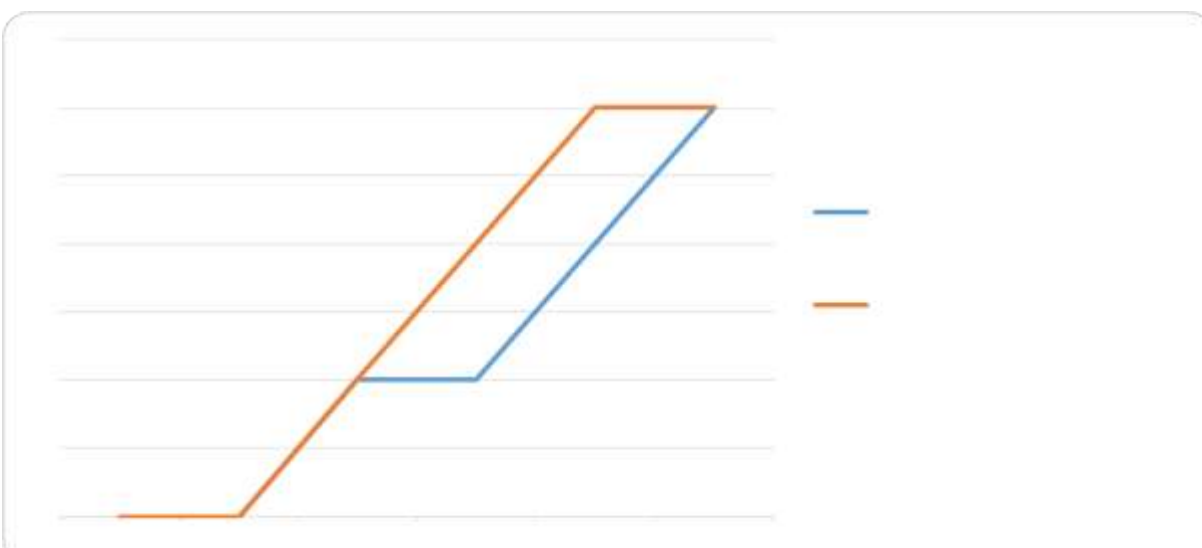

Figure 10: *Time-based onset of P. porrigens mycelial growth on PET-based fruiting substrates.*

4. DISCUSSION

5.1. Characteristics of *P. porrigens* Mycelium After Incubation

The incubation results of *P. porrigens* mycelium align with previous observations in *Pleurotus ostreatus*, which produces dense, cottony, white mycelial growth on culture media such as PDA under optimal conditions (Nashwan, 2021). This characteristic morphology may be attributed to strict aseptic handling and the use of selective Sabouraud agar supplemented with chloramphenicol, which effectively limits bacterial contamination.

The almost linear growth rate observed on enriched PDA (1.59 cm/day, R^2 = 0.9877) indicates efficient nutrient assimilation. Variations in growth rates between different *Pleurotus* species and substrates have been widely documented, with mycelial running and density dependent on both substrate composition and nutrient availability in the medium (Karmani et al., 2022; Maseko et al., 2024; Wang et al., 2025).

These findings reinforce that medium composition and strain-specific physiological traits play pivotal roles in determining mycelial growth dynamics in oyster mushrooms (Silva et al., 2025).

5.2. Comparison of Nutrient Solutions in Liquid Culture Spawn Production

Mean rank analyses (Tables 3 and 4) indicate that both substrate type and the presence of iodized salt significantly influenced *P. porrigens* mycelial growth. In salted media, the honey-based substrate (A3) promoted the earliest onset of white appearance (BWA = 3.00[a]) and rapid colonisation (MSC = 3.00[a]). In contrast, simple carbohydrate substrates (A1, A2, A4) and the salted lignocellulosic substrate (A5) exhibited delayed BWA and MSC, with differences statistically significant ($p < 0.05$).

In unsalted media, carbohydrate-based substrates (A'1–A'4) showed similar BWA values (2.00[b]), whereas the lignocellulosic substrate (A'5) had the earliest BWA (1.00[a]). Regarding MSC, A'1, A'2, and A'4 exhibited slower colonisation (3.00[c]); honey-based medium (A'3) showed intermediate performance (2.00[b]), and A'5 demonstrated the fastest colonisation (1.00[a]). The p-values obtained for BWA (0.046) and MSC (0.017) confirm the statistical significance of these differences.

Honey, rich in glucose (~38%), fructose (~31%), proteins, and other bioactive compounds, generally supports rapid mycelial growth (Karmani et al., 2022). However, the reduced growth observed in some honey-based media may not solely reflect osmotic effects from added salts but could also be influenced by the quality of the honey itself. Darkening of honey upon heating or prolonged storage suggests partial caramelisation, which may reduce the availability of sugars for fungal growth, though analytical confirmation is needed.

Overall, honey-based media, particularly those without iodized salt, tended to optimise rapid and uniform liquid spawn production of *P. porrigens*, highlighting the importance of nutrient-rich substrates and controlled osmolarity in fungal liquid culture systems (Maseko et al., 2024; Wang et al., 2025).

5.3. Evaluation of Liquid Spawn Viability on Crushed Maize Cobs

Mycelium cultivated on crushed maize cob substrates exhibited coloration ranging from white to pale yellow-orange and a cottony texture, consistent with general patterns observed in oyster mushroom substrates (Silva et al., 2025).

Variations in mycelial colour and texture across substrates may reflect differences in nutrient release, aeration, and substrate structure rather than contamination. The emergence of mycelium through PET bottle perforations confirms active colonisation prior to fructification.

Among the different spawn types tested, honey-based liquid spawn demonstrated high colonisation efficiency, likely due to the high content of readily utilizable sugars and other growth-promoting compounds inherent to honey. This observation aligns with studies showing that biological supplements can enhance mycelial growth when appropriately integrated into substrates (Nashwan, 2021; Maseko et al., 2024; Wang et al., 2025).

Overall, liquid spawn, particularly honey-based formulations, ensured faster and more homogeneous substrate colonisation than solid spawn, likely owing to more effective dispersion and immediate adherence of mycelial fragments upon inoculation (Karmani et al., 2022).

5. CONCLUSION

This study aimed to produce liquid spawn of *Pleurocybella porrigens* and to evaluate its viability on crushed maize cob substrates. Mycelium grown on PDA supplemented with chloramphenicol exhibited a whitish, cottony texture and a linear growth pattern, with an average rate of 1.59 cm/day ($Y = 1.406x + 1.094$, $R^2 \approx 1$). All liquid spawn produced from glucose-, fructose-, sucrose-, and honey-based media were viable. Among these, honey-based media supported the fastest mycelial growth and the earliest substrate colonization, likely due to their richness in simple sugars, enzymes, and essential minerals.

The addition of iodized salt resulted in slightly slower growth rates but promoted higher mycelial density and improved structural organization. Furthermore, visual darkening observed in honey-based media may have been associated with partial caramelization of the honey rather than chemical degradation.

When applied to crushed maize cob substrates, liquid spawn-particularly that derived from honey-based media-achieved rapid and dense colonization, readily visible through substrate pores. In contrast, solid spawn exhibited slower and less uniform colonization patterns.

Future research should focus on evaluating fructification performance according to spawn type, investigating the effects of raw versus caramelized honey on mycelial development, testing additional fruiting substrates, and incorporating genetic variability among strains in nutritional and productivity studies.

6. CONFLICT OF INTEREST

The authors declare that they have no competing interests.

7. AUTHORS' CONTRIBUTIONS

Study design: Nana Paulin, Eyamo Evina Victor Jos, and Claude Eugène Ebeh; **data collection and analysis:** Claude Eugène Ebeh, **draft writing:** All authors; **manuscript approval:** All authors

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