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Evaluation of substrate formulations for the reproductive structures production of entomopathogenic fungi of the family Cordycipitaceae (Hypocreales, Ascomycota)

Evaluación de formulaciones de sustrato para la producción de estructuras reproductivas de hongos entomopatógenos de la familia Cordycipitaceae (Hypocreales, Ascomycota)

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Abstract

The production of reproductive structures of entomopathogenic fungi was evaluated using strains isolated from the province of La Convención, Cusco, Peru: *Beauveria bassiana* (KIT-21), *Cordyceps* spp. (ALF-01), and two strains of *Isaria spp*. (IV-09 and PR-02). Four solid substrate formulations based on brown rice were incubated under controlled conditions, with the developmental phase of the reproductive structures occurring under blue LED light at 20 °C and 88% relative humidity. Synnemata and stromata length and fresh biomass were measured as

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response variables at 30 and 120 days, respectively. Because the data showed deviations from normality and heteroscedasticity, inferential analysis was conducted using a two-way ANOVA with Welch's correction, complemented by non-parametric tests (Kruskal-Wallis), which revealed significant effects of the substrate and substrate-strain interactions. Comparatively, the reproductive structures of *Isaria* showed the highest values. In particular, the greatest mean synnemata length was recorded in formulation F3, with PR-02 (26 mm) standing out, followed by F4 (25 mm), while IV-09 showed its best performance in F2 (25.7 mm). Fresh biomass followed a similar trend, with higher values observed for *Isaria*, especially PR-02 in F3 (1.14 g). Overall, the results indicate that brown rice substrate supplemented according to formulation F3 constitutes a viable and scalable substrate for the production of reproductive structures.

Keywords: *Beauveria bassiana; Cordyceps*; *Isaria*; Synnema; Stroma; Substrate.

Resumen

Se evaluó la producción de estructuras reproductivas de hongos entomopatógenos utilizando cepas aisladas de la provincia de La Convención, Cusco, Perú. *Beauveria bassiana* (KIT-21), *Cordyceps* spp. (ALF-01) y dos cepas de *Isaria* spp. (IV-09 y PR-02). Se utilizaron 4 formulaciones de sustrato sólido a base de arroz integral incubados en condiciones controladas y con fase de desarrollo de las estructuras reproductivas bajo luz LED azul a 20 °C y 88 % de humedad relativa. Como variables de respuesta se midieron la longitud y la biomasa fresca a los 30 y 120 días de los sinemas y estromas, respectivamente. Los datos mostraron desviaciones de la normalidad y heterocedasticidad; por lo tanto, el análisis inferencial se basó en un ANOVA de dos vías con corrección de Welch, complementando con pruebas no paramétricas (Kruskal-Wallis), evidenciando efectos significativos del sustrato y su interacción. Comparativamente, las estructuras reproductoras de *Isaria* mostraron los valores más altos. En particular, las mayores longitudes promedio de sinemas se registraron en la formulación F3, destacando PR-02 (26 mm) seguido de F4 (25 mm), mientras que IV-09 mostró su mejor desempeño en F2 (25,7 mm). La biomasa fresca siguió una tendencia similar, observándose valores más altos con *Isaria,* en especial PR-02 en F3 (1,14 g). La biomasa fresca siguió una tendencia similar, observándose valores más altos con *Isaria,* en especial PR-02 en F3 (1,14 g). En conjunto, los resultados indican que el arroz integral, suplementado según la formulación F3, constituye un sustrato viable y escalable para la producción de estructuras reproductivas.

Palabras clave: *Beauveria bassiana*; *Cordyceps*; *Isaria*; Estroma; Sinema; Sustrato.

INTRODUCTION

Entomopathogenic fungi such as *Beauveria bassiana*, *Cordyceps* spp., and *Isaria* spp. have gained relevance in sustainable agriculture as ecological alternatives to synthetic pesticides, reducing toxic residues while providing effective biological control (Angelo *et al*., 2025). In addition to their biocontrol potential, these fungi produce diverse secondary metabolites with insecticidal, antiviral, and antibacterial properties (Lucero *et al*., 2024). However, large scale application depends on efficient cultivation systems capable of producing high quantities of viable infective propagules. Both submerged and solid state culture methods are used, but solid state fermentation on low cost substrates is particularly suitable for industrial production (Hu *et al*., 2024). Among these, brown rice has been widely adopted due to its balanced nutrient composition, aeration capacity, and ability to retain moisture, supporting mycelial growth and sporulation (Taylor *et al*., 2013; Gutiérrez-Cárdenas *et al*., 2024). It has been shown to outperform other grains in promoting reproductive structures such as synnemata and stromata (Méndez *et al*., 2010), with studies reporting high sporulation and viability in species such as *Isaria tenuipes*, *Isaria fumosorosea*, and *Lecanicillium lecanii* when grown on rice-based substrates (Castillo *et al*., 2013; Cortez-Madrigal, 2007).

Fungal development is strongly influenced by physicochemical conditions, including substrate composition, temperature, humidity, and light (Gutiérrez-Cárdenas *et al*., 2024). Optimal growth and sporulation generally occur between 20 to 30 °C, although specific responses vary among species and strains (Tang, 2001; Céspedes *et al*., 2008). High relative humidity is essential for conidial germination and sporulation, with near saturated conditions maximizing yield (Luz & Fargues, 1999). Substrates such as brown rice help maintain these conditions due to their water retention capacity (Godoy *et al*., 2007; Arrubla *et al*., 2010). Light also plays a critical role in fungal morphogenesis, influencing pigmentation, structure formation, and sporulation, with blue light often enhancing reproductive development and enhanced bioinsecticide potency (Shrestha *et al*., 2006; Lucero *et al*., 2024).

Due to the relevance of brown rice-based substrates and the influence of physicochemical factors on fungal development, this study aimed to evaluate the growth and development of reproductive structures of entomopathogenic fungi belonging to the family Cordycipitaceae, cultivated on enriched brown rice supplemented with nutrient solutions under controlled conditions. Different nutrient formulations were tested to assess their effect on the formation of reproductive structures (e.g., synnemata and stromata), using strains collected from diverse ecological zones in Cusco.

MATERIALS AND METHODS

Fungal strains and maintenance

The strains were collected in the Province of La Convención-Cusco, in the districts of Echarati and Huayopata, in the populated centers of Kiteni, Ivochote, Palma Real and Alfamayo. With the corresponding codes KIT-21 (12°39' 31.2" S, 73°01'11.2" W, altitude 1218), IV-09 (12°27' 24.3"S, 73°57' 59.9"W, altitude 571m.), PR-02 (12°37'31.6"S, 72°41'46.1"W, altitude 742m), ALF-01 (13°03' 40.4"S, 72°24'24.7"W, altitude 2519m.). After collection, the samples were transported to the Center for Research and Production of Food and Medicinal Mushrooms (CIPHAM) at the National University of San Antonio Abad of Cusco (UNSAAC) for isolation and purification. Before experimentation, each strain was reactivated by suspension in sterile 0.9% (w/v) sodium chloride to restore viability and metabolic activity prior to inoculation into the test substrates.

Substrate formulations and sterilization

The solid base substrate consisted of whole brown rice (*Oryza sativa*), which was hydrated and enriched with nutrient solutions prepared in distilled water. Four experimental formulations were evaluated, differing in their sources of carbon, nitrogen, vitamins, and minerals. Formulation 1 (F1) contained dextrose, malt extract, yeast extract, peptone, cassava starch, seaweed powder, and gypsum; F2 combined cassava starch, magnesium sulfate, peptone, gypsum, seaweed powder, vitamin B12, and monopotassium phosphate; F3 included a whole egg along with malt and yeast extracts; and F4 consisted of an oat (*Avena sativa*) infusion (Table 1). The substrates were distributed into 0.23 L and 1.0 L polypropylene containers with modified lids to allow controlled gas exchange and aseptic access. Rice-to-solution ratios were adjusted for each formulation to ensure uniform nutrient distribution. The filled containers were sterilized in an autoclave at 121 °C for 20 minutes and cooled to room temperature before inoculation.

Inoculation, incubation and harvesting

Mycelial suspensions were prepared in sterile 0.9% (w/v) sodium chloride and used as inoculum. Under aseptic conditions in a laminar flow cabinet, 4 mL of each suspension were aseptically pipetted into separate containers for the four formulations to minimize microbial contamination. The cultures were first incubated in complete darkness at 21 ± 1 °C for 30 days to allow complete colonization of the substrate (0.23 L). Once colonized, the material was aseptically transferred to 1.0 L containers within a laminar flow cabinet. Reproductive structure development was then induced in a

Table 1. Composition of experimental substrate formulations.

Tabla 1. Composición de las formulaciones de los sustratos experimentales.

Substrate	Nutrient solution composition	Base solid substrate: nutrient solution ratio
F1	10 g dextrose, 10 g malt extract, 5 g yeast extract, 4 g peptone, 10 g cassava starch, 3 g algal powder, 1 g gypsum; made up to 1 L with distilled water	20 g brown rice + 30 mL solution
F2	2.5 g cassava starch, 1.25 g $MgSO_4$, 4.75 g peptone, 0.875 g gypsum, 2.75 g algal powder, 2.5 g vitamin B_{12}, 0.0625 g KH_2PO_4; made up to 1 L with distilled water	28 g brown rice + 30 mL solution
F3	1 whole egg, 28.35 g malt extract, 28.35 g yeast extract; made up to 1 L with distilled water	35 g brown rice + 30 mL solution
F4	60 g oats (Avena sativa); made up to 1 L with distilled water	35 g brown rice + 30 mL solution

modified incubator providing diffuse blue LED light (~65 lux) under a 12 h light/12 h dark cycle, 88% relative humidity (Fig. S1), and a constant temperature of 20 °C (Fig. 1). This phase lasted 90 days to promote sporulation and the formation of reproductive structures (stroma and synnema).

Macroscopic development process was recorded in two sequential phases. In the first phase, the complete colonization of the substrates was monitored, with detailed evaluation of the mycelium's morphology and pigmentation (Fig. 1). In the second phase, after 120 days of incubation (Fig. 2), the reproductive structures were aseptically collected to determine their length and fresh weight using an analytical balance. Finally, the samples were dehydrated under controlled conditions and stored at 4 °C.

Data handling and statistical analysis

Synnemata length and biomass were screened for outliers using 1.5×IQR and 3.5×MAD. For biomass, we calculated the median, 10% trimmed mean, winsorized mean, and post-outlier mean, adopting the latter as the operative average and for normalizing synnemata length to minimize treatment-level biomass effects. Assumption testing indicated significant deviations from normality (Shapiro–Wilk, Kolmogorov–Smirnov, Lilliefors, and Jarque–Bera) and unequal variances among groups (Levene's test). Consequently, robust and nonparametric methods were used. The main comparison employed a two-way Welch ANOVA with heteroscedasticity-consistent covariances, complemented by a Kruskal–Wallis test for overall group effects. Pairwise contrasts were performed on estimated marginal means using Wilcoxon tests with Tukey adjustment and Holm or Benjamini–Hochberg corrections to control type I error. Effect sizes

were reported as eta-squared and its nonparametric analog (η^2[H]) for Kruskal–Wallis. The association between synnemata length and biomass was assessed using Spearman's correlation. All analyses were performed in R with tidyverse for data handling (Wickham *et al*., 2019), *ggplot2* and *ggpubr* for visualization (Kassambara, 2026), *car* for assumption testing and heteroscedastic adjustments (Fox *et al.*, 2026), *WRS2* for robust methods (Mair & Wilcox, 2020), *rstatix* for nonparametric workflows (Kassambara, 2025), *emmeans* for estimated marginal means and contrasts, and *effectsize* for effect size estimation (Lenth & Piaskowski, 2026).

RESULTS

Macroscopic growth diverged early among strains. The two *Isaria* strains (IV-09 and PR-02) rapidly and uniformly colonized the brown rice substrate, achieving near-complete surface coverage after about 20 days in darkness. In contrast, *Beauveria bassiana* (KIT-21) and *Cordyceps* sp. (ALF-01) to achieve complete substrate coverage. Distinct mycelial morphologies were consistently observed, with minor variation among formulations. *Isaria* cultures produced abundant cottony white mycelia that became firmer and developed pale yellow tones upon maturation, whereas *B. bassiana* formed dense white mycelium that became drier during conidiation, showing orange pigmentation associated with spore masses. *Cordyceps* (ALF-01) exhibited a more uniform texture, transitioning from light pink in early stages to deeper reddish-brown tones at maturity.

These qualitative traits across strains and substrate formulations are illustrated at different developmental stages (Fig. 1), with additional details on mycelial color, texture, density, and surface characteristics for media F1–F4 provided in Table S1. The development of reproductive structures began during the second incubation phase, with synnemata (in *Isaria*) and stromata (in *Cordyceps*) becoming visible around day 60, although growth rates and morphology varied among strains and substrates. By day 120, these structures were fully developed. Synnemata (in *Isaria*) and conidia-bearing structures (in *B. bassiana*) were then collected, and synnema length and fresh biomass were measured (Fig. 2). Yields for each strain–substrate combination are summarized in Table 2. Overall, *Isaria* sp. PR-02 showed the most consistent productivity across media, yielding high biomass in formulations F2–F4. *Isaria* sp. IV-09 reached its greatest mean synnema length in F2. *B. bassiana* (KIT-21) produced shorter structures, while *Cordyceps* sp. (ALF-01) exhibited the lowest productivity, forming relatively small stromata. PR-02 achieved the highest output with ~1.14 g fresh biomass in F3, whereas ALF-01 produced <0.5 g in all cases. In general, PR-02 and IV-09 developed the longest synnemata (20–26 mm depending on medium), contrasting with the much shorter structures observed in KIT-21 and ALF-01.

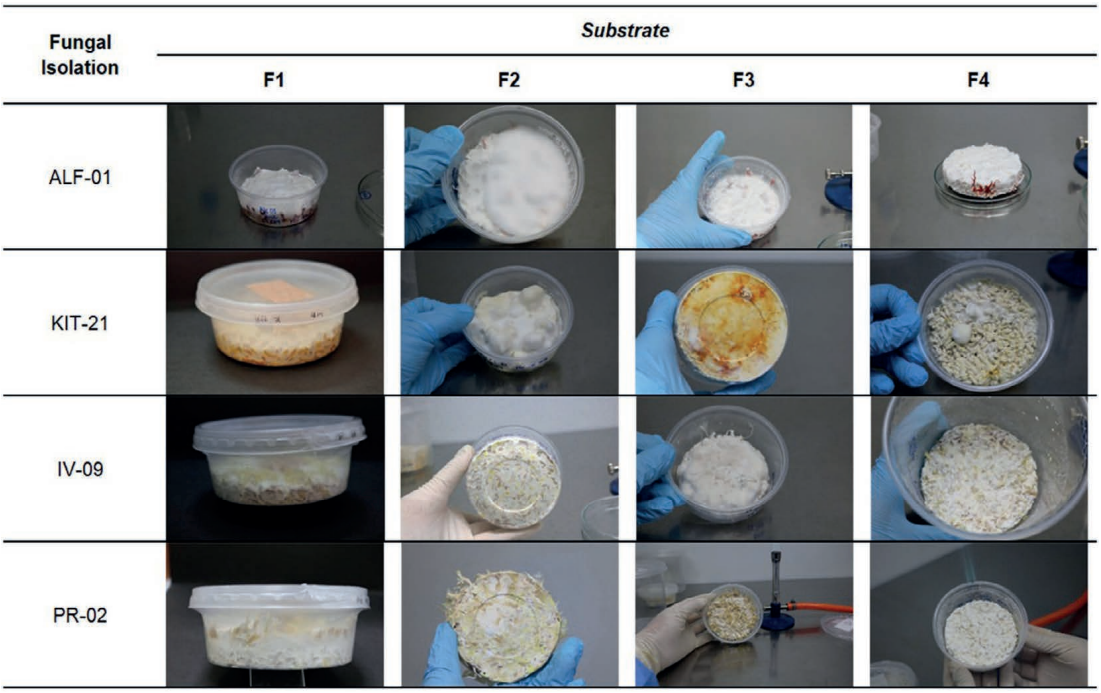

Fig. 1. Mycelial growth by strain, developed during the first incubation stage up to 30 days.

Fig. 1. Crecimiento micelial por cepa, desarrollado durante la primera etapa de incubación hasta los 30 días.

Table 2. Reproductive structures length according to strain biomass.

Tabla 2. Longitud de estructuras reproductivas según biomasa de la cepa.

Fungal Isolation	Substrates	n	Mean	SD	Median	IQR	Min	Max	Avg Weight (g)
ALF-01	F1	74	15.0	4.69	15.0	5.88	5	24	0.11
ALF-01	F2	7	11.4	1.90	11.0	1.50	9	15	0.01
ALF-01	F3	130	11.0	3.59	10.0	5.75	3	20	0.10
ALF-01	F4	16	10.4	4.30	10.5	6.00	3	20	0.03
IV-09	F1	330	17.9	8.07	16.2	10.50	5	40	0.84
IV-09	F2	994	25.7	10.90	25.0	15.90	5	55	0.32
IV-09	F3	168	23.3	12.20	22.0	15.50	3	49.5	0.91
IV-09	F4	955	22.6	9.98	20.0	11.30	3	52	0.44
KIT-21	F1	8	8.12	4.19	7.0	4.25	1	15	0.10
KIT-21	F2	2	20.5	6.36	20.5	4.50	16	25	1.25
KIT-21	F3	18	20.6	8.70	18.2	15.0	11.5	35.5	0.13
KIT-21	F4	42	16.7	6.05	15.0	10.0	10	32	0.24
PR-02	F1	82	19.5	8.75	20.8	13.40	5	37	0.62
PR-02	F2	625	22.6	9.51	20.5	12.50	5	52	1.13
PR-02	F3	162	26.0	10.90	25.0	17.50	5	55	1.14
PR-02	F4	1058	25.0	11.90	23.0	19.00	4	55	0.85

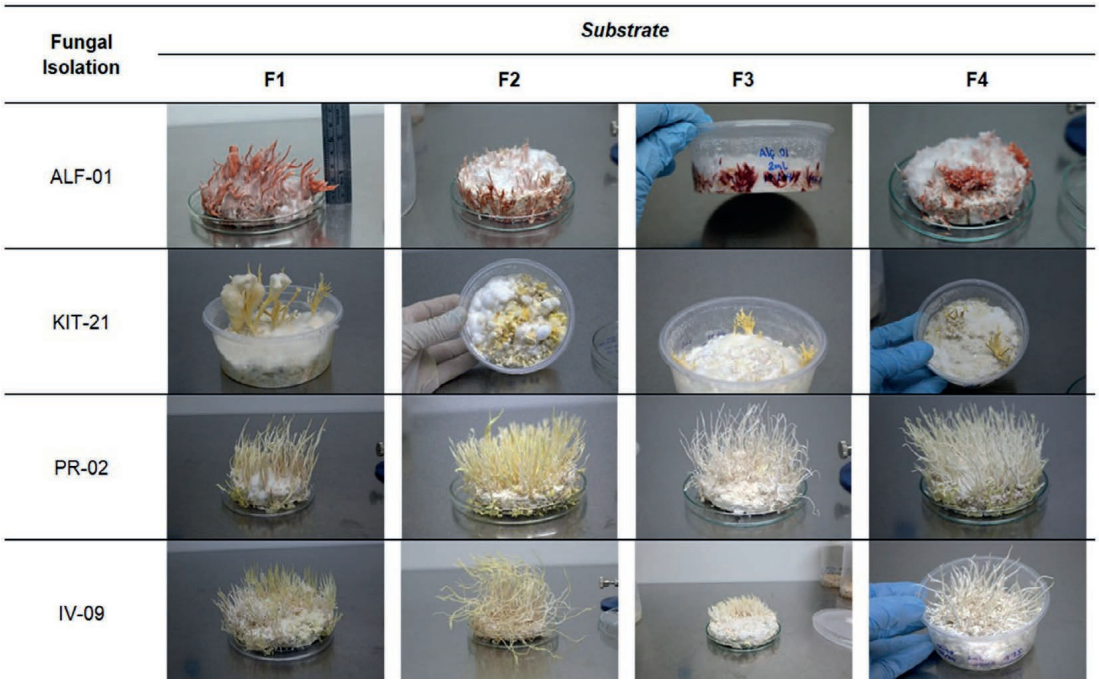

Fig. 2. Growth of reproductive structures during the second incubation stage at 120 days.

Fig. 2. Crecimiento de estructuras reproductivas durante la segunda etapa de incubación hasta los 120 días.

Statistical analyses indicated that synnema length data violated the assumptions of normality and homogeneity required for ANOVA. The Shapiro–Wilk ($W = 0.966$, $p < 0.001$), Kolmogorov–Smirnov ($D = 0.081$, $p < 0.001$), Lilliefors ($p < 0.001$), and Jarque–Bera ($\chi^2 = 273.64$, $p < 0.001$) tests indicated a non-normal distribution, while Levene's test revealed heteroscedasticity ($F = 36.66$, $p < 0.001$). Therefore, a two-way Welch ANOVA, robust to unequal variances, was used to evaluate the effects of culture medium, fungal strain, and their interaction on synnema length. The analysis revealed highly significant effects of medium ($F = 19.24$, $p < 0.001$) and strain ($F = 428.93$, $p < 0.001$), as well as a significant substrate–strain interaction ($F = 32.76$, $p < 0.001$), with a moderate effect size ($\square^2 \approx 0.105$). The results were confirmed by a nonparametric Kruskal–Wallis test ($\chi^2 = 563.28$, $p < 0.001$), which reinforced the existence of differences among the 16 strain–substrate combinations. In complementary one-way analyses (Fig. 3), the ANOVA with pairwise *t* comparisons adjusted by the Benjamini–Hochberg method showed that substrate F2 > F1, F3, and F4 (all $p < 2.22e{-}16$), F2 > F3 ($p = 0.04$), and F2 > F4 ($p = 0.019$), while F3 and F4 did not differ ($p = 0.87$) (Fig. 3A). For fungal strain, IV-09 (*Isaria* sp.), KIT-21 (*Beauveria bassiana*), and PR-02 (*Isaria* sp.) all exceeded *Cordyceps* ALF-01 (all $p < 2.22e{-}16$). Moreover, PR-02 > IV-09 ($p \approx 2.1e{-}07$), PR-02 > KIT-21 ($p \approx 1.5e{-}12$), and IV-09 > KIT-21 ($p \approx 7.7e{-}10$) (Fig. 3B). The combination-level comparisons (Fig. 3C) maintained a globally significant effect ($p < 2e{-}16$) and consistently

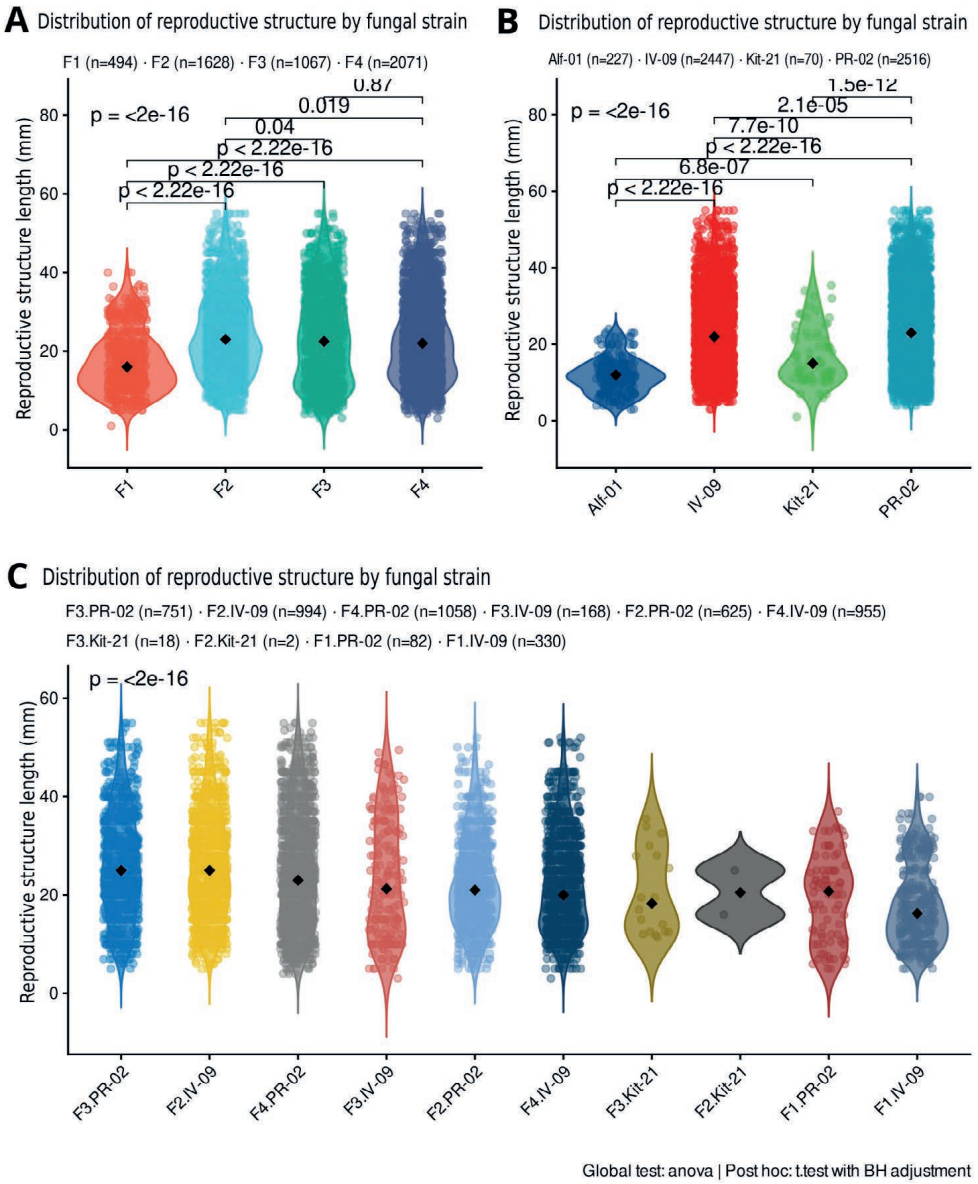

Fig. 3. A) Length of reproductive structures across substrates. B) Reproductive structure length across fungal strains. C) Top 10 substrate and strain combinations ordered by mean length of reproductive structures. Dots represent individual observations; violins, the distribution; and the black diamond, the median. Global tests: one-way ANOVA; post hoc: pairwise t-tests with Benjamini–Hochberg (BH) adjustment.

Fig. 3. A) Longitud de estructuras reproductivas en los sustratos. B) Longitud de estructuras reproductivas en las cepas fúngicas. C) Las 10 principales combinaciones de sustrato y cepa, ordenadas por longitud media de estructuras reproductivas. Los puntos muestran las observaciones individuales, los violines la distribución y el diamante negro la mediana. Pruebas globales: ANOVA de una vía; post hoc: pruebas t por pares con ajuste de Benjamini-Hochberg (BH).

placed F3–PR-02, F2–IV-09, F4–PR-02, and F3–IV-09 among the highest in synnema length, with significant differences compared to combinations involving ALF-01 and several involving KIT-21 after Benjamini–Hochberg adjustment. Post hoc comparisons revealed distinct patterns: *Isaria* PR-02 in F3 produced the longest synnemata (~26 mm), followed by IV-09 in F2 (~25.7 mm) and PR-02 in F4 (~25 mm). In contrast, *Cordyceps* ALF-01 formed the shortest structures (<15 mm across all media), while *B. bassiana* KIT-21 showed strong medium dependence, with moderate lengths in F2–F3 (~20.5 mm) but reduced growth in F1 (~8.1 mm).

DISCUSSION

This study shows that both the fungal strain and the composition of the solid-state substrate clearly affect growth and reproductive performance of entomopathogenic fungi under controlled conditions. The *Isaria* strains (IV-09 and PR-02) consistently outperformed B. bassiana (KIT-21) and *Cordyceps* sp. (ALF-01), particularly on nutrient-enriched rice media, supporting previous evidence that productivity varies markedly among species and culture conditions (Yadav *et al*., 2013). The faster substrate coverage observed in *Isaria* (~20 days vs. ~30 days) suggests a more aggressive saprobic strategy. Many *Isaria* species, historically classified as *Paecilomyces* and currently subject to taxonomic revision within Cordycipitaceae, are characterized by rapid proliferation on grain-based substrates and the formation of synnemata under favorable conditions (Lee *et al*., 2008, Liu *et al*., 2019). In contrast, *Beauveria bassiana* typically exhibits a more diffuse and comparatively slower mycelial expansion on solid substrates, reflecting differences in growth dynamics and developmental strategies during early substrate colonization (Garza-López *et al*., 2011; Toledo *et al*., 2008). Consistent with this, KIT-21 formed dense, powdery colonies, whereas Isaria strains developed aerial mycelia that differentiated into thick synnemata with pigmented apices. Pigmentation patterns further reflected known physiological traits. The pale yellow coloration in *Isaria* synnemata agrees with previous reports of maturation-associated spore pigmentation (Yadav *et al*., 2013) while the gradual reddening observed in *Cordyceps sp*. ALF-01 likely corresponds to carotenoid production, as described for *Cordyceps militaris* (Feng *et al*., 2018). Light is a key regulator of this process: stromata formation and pigmentation in *Cordyceps* depend strongly on illumination, with blue light enhancing both development and yield (Shrestha *et al*., 2006, Wu *et al*., 2023). Therefore, the limited performance of ALF-01 in this study was likely constrained by suboptimal light conditions, highlighting the need to optimize photoperiod and light quality in future work.

Effects of substrate formulation

The substrate or culture medium had a strong influence on fungal performance, underscoring the importance of both nutritional and physical conditions for large-scale cultivation. Formulation F3 yielded the longest reproductive structures and highest biomass in several strains, indicating its suitability for promoting fungal growth. Rice-based substrates are widely used in commercial production systems for *Beauveria, Isaria, and Metarhizium* due to their favorable nutrient profile and moisture retention capacity (Yadav *et al*., 2013). In particular, brown rice provides vitamins, minerals, and complex carbohydrates that can enhance mycelial growth and sporulation (Ji & Ra, 2021). Several studies report higher conidial yields on brown rice compared to other grains such as wheat, corn, or sorghum (Vimala Devi *et al*., 2001; Castillo *et al*., 2013). However, not all studies support the superiority of brown rice. For example, Rangel *et al*. (2023) reported higher conidial production on polished rice for several fungi, including *B. bassiana* and *Metarhizium*. Similarly, broken rice has been identified as an even more productive substrate, likely due to increased surface area and improved moisture distribution, with yields reaching 10^{1}–10^{11} conidia per gram for *B. bassiana* and *M. anisopliae* (Madhavi *et al*., 2020; Yadav *et al*., 2013). These findings suggest that substrate performance depends not only on nutrient composition but also on physical properties such as structure and water availability. In our study, the superiority of formulation F3 over F1 (which produced the lowest results) aligns with these observations: a nutritionally rich and well-structured substrate is essential for maximizing growth. Developing cost-effective and reliable substrates remains a major bottleneck for scaling up mycoinsecticide production (Yadav *et al*., 2013). Accordingly, we found that the choice of substrate and fungal strain significantly influences vegetative growth and reproduction. This highlights their combined role in the morphogenesis and productivity of entomopathogenic fungi.

Fungal strain-specific performance

The effect of fungal strain on performance evidences the role of genetic variability. Even within a single species or genus, strains can differ substantially in growth rate, sporulation capacity, and environmental tolerance. For example, Rangel *et al*. (2023) reported nearly an order of magnitude difference in conidial yield between two *B. bassiana* strains grown on the same substrate. In our study, both *Isaria* strains were highly productive, though PR-02 consistently outperformed IV-09 in rep body biomass. These differences likely reflect intrinsic variation in metabolic efficiency or stress tolerance, underscoring the importance of strain selection (Pöggeler *et al*., 2018). In contrast, *Cordyceps* sp. ALF-01 was a low-performing strain across all parameters, probably due to

intrinsic factors (e.g., a slow-growing species or specific fructification requirements) or suboptimal experimental conditions (such as lack of light or other necessary stimulant, as previously discussed). *Beauveria* exhibited a marked dependence on substrate formulation, performing well in F2 but poorly in F1. This suggests that its growth is particularly sensitive to specific nutritional or physical factors, F2 may have provided a more suitable C:N ratio or moisture level for *B. bassiana*, whereas F1 lacked them. The significant strain–substrate interaction observed in this study reinforces that fungal responses are highly context-dependent, requiring tailored combinations of strain and substrate. Practically, this implies that for large-scale production, multiple strains should be evaluated across different substrates to identify the most efficient combination. Rice performed well for *Isaria* in this study, but a *Cordyceps* strain might respond better to rice media supplemented with insect-derived nutrients such as silkworm pupae or *Galleria mellonella* powder, which are used in some protocols to induce stroma formation (Reyes-Haro, 2018). Similarly, *B. bassiana* could benefit from small adjustments in temperature or pH to stimulate uniform sporulation (Lucero *et al*., 2024). Therefore, when evaluating productive strains, both morphological and quantitative parameters should be considered. Moreover, the relationship between these variables could be improved by adjusting environmental factors such as humidity and aeration without compromising spore yield (Reyes-Haro, 2018). High relative humidity (close to 100% RH) is particularly important for maximizing conidiation in *B. bassiana* and other entomopathogenic fungi, as it promotes both conidial germination and sustained development of sporulating structures during the reproductive phase (Rangel *et al*., 2023; Yadav *et al*., 2013), ensuring that dense mycelial layers remain active and continue sporulating rather than drying out. Overall, the superior performance of *Isaria* in our trials is promising, since species of this genus (e.g., *Isaria fumosorosea* and *I. tenuipes*) are widely used in biological control and even have pharmaceutical value (Chhetri *et al*., 2020). Meanwhile, *Beauveria bassiana*, although a widely employed biocontrol agent, typically requires careful optimization of culture conditions to achieve comparable spore yields; however, as shown in several studies, it can reach very high productivity under ideal conditions (Rangel *et al*., 2023). The limited performance of *Cordyceps sp*. ALF-01 suggests that further refinement of cultivation conditions is necessary to fully exploit its potential, particularly given the biotechnological value of related species such as *Cordyceps militaris*.

CONCLUSIONS

In conclusion, this study demonstrates that both organism selection and substrate formulation must be carefully optimized to ensure successful cultivation. By employing high-performing strains (such as *Isaria* PR-02 in this case) and adjusting substrate and environmental parameters, such as nutrient-enriched rice media, high humidity, and appropriate light regimes, it is possible to notably enhance biological production efficiency. These improvements hold not only scientific value but also practical importance, enabling more cost-effective large-scale production of biocontrol agents and promoting the broader adoption of eco-friendly mycopesticides in sustainable agriculture.

Among the evaluated substrates, formulation F3 showed superior performance, producing the longest synnemata and highest biomass across several strains, followed by F2, while F1 yielded the lowest results. Statistical analyses confirmed significant main effects of both substrate and strain, as well as a strong interaction between them, indicating that fungal response depends on the specific combination of strain and medium. The strong performance of *Isaria* on grain-based substrates and the high yields reported for *Beauveria* on optimized rice media reaffirm that solid-state fermentation remains a viable and scalable approach for entomopathogenic fungi production. Continued research should focus on refining cultivation methods, testing alternative substrates or agroindustrial by-products, and elucidating the genetic basis of high-yield strains, all crucial steps toward improving the accessibility and effectiveness of fungal-based biocontrol solutions.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Table S1. Qualitative macromorphology during the first 30 days of incubation by strain and formulation (color, texture, density, surface features).

Tabla S1. Macromorfología cualitativa durante los primeros 30 días de incubación por cepa y formulación (color, textura, densidad, características superficiales).

Fungal strain	F1	F2	F3	F4
KIT-21 (*Beauveria bassiana*)	White, dense, cottony mycelium; upon maturation it develops a powdery surface with irregular tones from white to orange, with yellowish to reddish hues.	White, cottony, dense mycelium; upon maturation it shows an irregular texture with intense orange patches and prominences up to 8 mm.	White, cottony, very dense mycelium; upon maturation it becomes powdery with chromatic variation toward orange, with white areas and yellowish to reddish hues.	White mycelium, scarce and irregular; upon maturation it presents a powdery surface with orange patches and small white-cream aggregates up to 2 mm.
PR-02 (*Isaria* spp.)	White, cottony, very dense mycelium; upon maturation it shows scattered patches with a faint yellow hue.	White, cottony, irregular mycelium; small elevations are observed and a faint yellow coloration on maturation.	Very dense, irregular cottony mycelium with localized elevations; cream to white in color.	White, scant mycelium; upon maturation it becomes powdery with small aggregates.
IV-09 (*Isaria* spp.)	White, cottony, very dense mycelium; upon maturation it presents scattered pale-yellow patches.	White, cottony, very dense mycelium with accelerated colonization; upon maturation it retains pale-yellow patches.	High-density, irregular cottony mycelium with localized elevations; cream to white in color.	White, scant mycelium; upon maturation it develops a powdery surface with small aggregates.
ALF-01 (*Cordyceps* spp.)	White, cottony, very dense mycelium with a regular structure; upon maturation it shows a color transition from light red to dark red.	Cottony, irregular mycelium with dense areas; predominantly white with orange and yellow patches; localized powdery texture.	White cottony mycelium with a regular structure; upon maturation it shifts from dark red to maroon.	White, irregular cottony mycelium with small aggregations; upon maturation it acquires a faint yellow coloration.

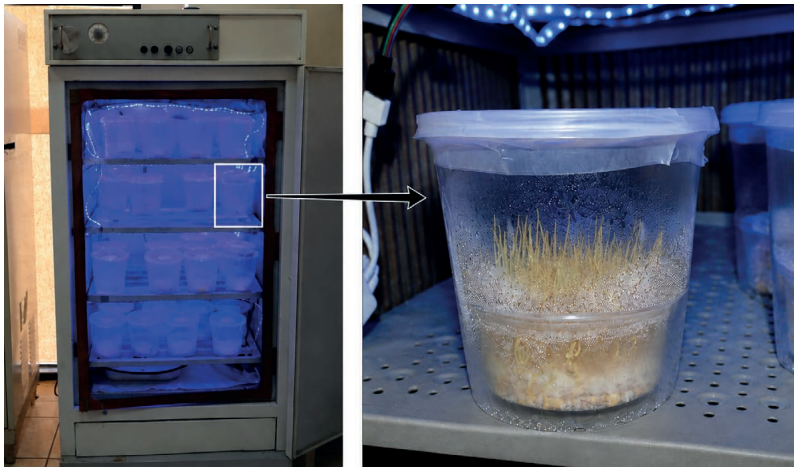

Fig. S1. Development of reproductive structures incubated for 120 days with blue LED light.

Fig. S1. Desarrollo de estructuras reproductivas incubadas durante 120 días con luz LED azul.