



Formulation of Spray-Dried *Cordyceps fumosorosea* Submerged Spores Containing Water-Soluble Sunlight Protectants

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Abstract

This research aimed to enhance the stability of *Cordyceps fumosorosea* spores (JKI-BI-1496) using spray-drying with water-soluble sunlight protectants. Submerged spores (ss) of JKI-BI-1496 were tested for survival after spray-drying. Humic acid Na, humic acid K, lignin, black tea, cocoa, coffee, skimmed milk, green tea, and calcofluor white were evaluated for integration into the spray-drying process. Absorbance profiles (280 - 800 nm) were examined. Spray-drying was conducted at 65 °C inlet, 48 °C outlet, and a maximal product temperature of 35°C. Skimmed milk powder was used as a protective agent, forming a suspension with 5% skimmed milk, 5×10^6 ss mL⁻¹ spores, and 5% protectants. Germination rates were assessed after 1 - 4 hours of simulated solar radiation. Spores coated with black tea showed the highest germination rate (48.3%) after 4 hours, compared to < 18% with other protectants. Calcofluor white-coated spores exhibited 44.9% germination after 12 weeks at 6 °C. Black tea and calcofluor white, enhance the stability of JKI-BI-1496 spores, indicating their potential for outdoor agricultural applications.

Keywords: *Cordyceps fumosorosea*; Water-soluble sunlight protectants; Spray-drying; Spore viability; Storability.

INTRODUCTION

Solar radiation, essential for life on Earth, includes ultraviolet (UV), visible, and infrared bands, each impacting the environment differently [1]. While UV radiation is a minor portion, it significantly influences biological processes, including gene expression, metabolism, and plant-insect interactions [2]. Of the sunlight reaching earth, infrared radiation (>700 nm) accounts for 49.4%, visible light (400-700 nm) for 42.3%, and UV (100-400 nm) just over 8% [1-3]. Sunlight also impacts the fungal life cycle, affecting reproduction, growth, and virulence [4]. UV-B specifically poses a challenge to entomopathogenic fungi (EPF), affecting spore survival, which is crucial for insect control in natural settings [5,6]. Studies show that artificial sunlight exposure significantly reduces survival in EPF species, with *Metarhizium flavoviride* showing the highest tolerance and *Cordyceps fumosorosea* the least [7]. For example, *Metarhizium spp.* conidia exhibited over 50% germination loss after two hours of UV-B exposure [8]. Prolonged exposure, such as six to eight hours, can render some isolates inactive. Due to the sensitivity of fungal propagules to UV-B, various formulations, including physical treatments, have been explored to enhance EPF resilience under sunlight [6].

Proper drying and formulation handling are essential for microbial biopesticide development, especially for blastospores [9]. Stephan and Zimmermann et al. [10], pioneered spray-drying for submerged spores

of *M. anisopliae* and *M. flavoviride*, achieving >80% viability post-drying. Progress has also been seen with *C. fumosorosea* and *B. bassiana*, showing high blastospore survival post-freezing, spray-, or air-drying [11-13].

This study's first objective was to develop a spray-drying method for submerged spores of *C. fumosorosea* (strain JKI-BI-1496). Selecting the right sunlight protectant coating was crucial, as oil-based formulations generally improve UV tolerance and germination more than water-based ones, though water-soluble protectants provide an evaporative protective layer [5-14]. While substances like calcofluor white show promise in controlled settings, field data remain limited [15,16]. Therefore, the second objective included screening water-soluble protectants based on their light absorbance as part of evaluating their potential to enhance spore stability under field-relevant conditions.

The third objective was to evaluate simulated sunlight exposure effects on *C. fumosorosea* spore germination, assessing the viability of protectant formulations for sunlight tolerance. Limited data exist on *C. fumosorosea* shelf life, though studies on *B. bassiana* blastospores reveal up to 90% viability under modified atmosphere storage [12-17]. Dry microgranular formulations are advantageous for shelf life, handling, and water-based agricultural sprays [12-14]. The fourth objective was to assess sunlight protectants' effect on spray-dried spore storability.

MATERIALS AND METHODS

Production of submerged spores of JKI-BI-1496

For this study, *C. fumosorosea* strain JKI-BI-1496, isolated from *Cydia pomonella* in 1971 by Müller-Kögler in Darmstadt, Germany (JKI Inventory), was used. This strain is effective against hosts like *Bemisia tabaci*, *C. pomonella*, *C. funebrana*, and *C. molesta* and there is interest in developing a biopesticides based on this strain. JKI-BI-1496 was sourced from JKI's collection, stored at -80 °C, and cultured on malt peptone agar (MPA) with 3% malt extract, 0.5% soybean peptone, and 1.8% agar. For experiments, the strain was cultivated in liquid culture using 100-mL Erlenmeyer flasks containing 50 mL autoclaved malt peptone broth (30 g/L malt extract, 9 g/L peptone). Flasks inoculated with 14-day-old conidia were incubated for 72 hours at 25 °C and 150 rpm on a horizontal shaker. Spore counts were conducted using a Thoma counting chamber. New starter cultures were prepared for each experiment, and inoculated media were derived from these cultures.

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For submerged spore production, modified S8 medium (25 g/L glucose, 20 g/L corn steep solid, 5 g/L sodium chloride) was inoculated with 1×10^6 ss/mL (ss= submerged spores) from the starter culture and cultivated for 72 hours under similar conditions, then adjusted to required spore numbers.

Production of spray-dried spores: Produced submerged spores were filtered through three layers of gauze and were centrifuged for 5 minutes at 3000 g. The resulting pellet was then re-suspended in deionized water. A suspension was prepared, containing 2×10^7 ss/mL submerged spores, using autoclaved water. A 20% (w/v) solution of skimmed milk was made with boiled autoclaved water. After that, spore suspension and solution of skimmed milk were combined in a 1:1 ratio, resulting in a 50 mL suspension containing 10% (w/v) skimmed milk and 1×10^7 ss/mL submerged spores. Subsequently, all spray-drying parameters of the spray-dryer (Mini spray dryer S-300, BÜCHI Labortechnik AG, Flawil, Switzerland) were adjusted (Supplementary Table 1). After spray-frying the powder containing submerged spores was taken out from the jar carefully and kept in sterile 25-mL Eppendorf conical tubes (Carl Roth GmbH + Co. KG, Karlsruhe, Germany).

Feasibility test

After spray-drying, the feasibility of spray-drying was tested by comparing the viability of not spray-dried and spray-dried spores. For this purpose, not spray-dried and spray-dried submerged spores were mixed thoroughly in 10 mL of autoclaved water. Three 10 μ L droplets from both sample were pipetted onto a MPA plate. All plates were then incubated at 20 °C for 16 hours, after which the samples were stained with Lactophenol blue (Carl Roth GmbH, Karlsruhe, Germany) and the germination rate was determined by counting 100 spores. A spore was considered germinated when the length of the germ tube equaled or exceeded the width of the spore. The experiment was repeated three times with three replications.

Selection of water-soluble sunlight protectants

The selection of water-soluble additives (Supplementary Table 2) with potential sunlight-protective effects was based on an earlier study [16]. For preparation of liquid stock solution, 20% (w/v) of humic acid Na, humic acid K, lignin and skimmed milk were mixed with autoclaved distilled water. Calcofluor white solution was also prepared with the same manner. 20% (w/v) of black tea, coffee, cocoa and green tea was taken and brewed with sterile distilled water at 70 °C for 15 minutes. After that samples were diluted again twice at 1:10 ratio for wavelength spectrum by spectrometer.

ABSORPTION MEASUREMENT

A FLUOstar® Omega spectrometer (BMG LABTECH, Ortenberg, Germany) was used to measure the absorption spectrum of nine

potential sunlight protectants. In each well of a 96-well plate (GREINER 96 U-BOTTOM, Greiner Bio-One GmbH, Frickenhausen, Germany), 100 μ L of sample was added, and ten flashes per well were captured to ensure thorough data acquisition. Measurements were taken across wavelengths from 280 to 800 nm with 2 nm steps and a 0.1-second settling time. This process was repeated three times, each with three replicates. Absorbance data was analyzed using MARS software, providing comprehensive data insights from the FLUOstar Omega reader.

Production of spray-dried submerged spores coated with sunlight protectants:

To produce spray-dried submerged spores coated with sunlight protectants, 50 mL suspension of submerged spores (containing 10% skimmed milk and 1×10^7 ss/mL submerged spores) has been prepared, as described before. In parallel, a 10% solution of selected sunlight protectants was prepared using autoclaved water. Afterwards, spore suspension and solution of sunlight protectants were also mixed in a 1:1 ratio (25 mL of 5% skimmed milk with 5×10^6 ss/mL submerged spores + 25 mL of 5% sunlight protectants). Then, resulting 50 mL suspension was turned into powder form by spray-drying following the same protocol as described earlier. Production of spray-dried submerged spores coated with sunlight protectants was repeated three times independently. The spray-dried spores were kept for maximal 24 hours at 6 °C before testing at simulated sunlight.

Sunlight simulation through Atlas XXL Sunlight simulator:

After production of submerged spores coated with sunlight protectants, all samples of spray-dried spores were exposed to simulated sunlight in a SUNTEST XXL + FD sunlight simulator (Atlas Material Testing Technology, Illinois, USA) for 1, 2, 3 and 4 hours. Therefore, the spray-dried spores were mixed thoroughly in 10 mL of autoclaved water. Subsequently, 1 mL of this mixture was added to each well of a 24-well plate (Cell culture multiwell plate 24 well, PS, Greiner Bio-One GmbH, Frickenhausen, Germany) for sunlight simulation exposition. Another multi-well plate, designated as a control, was covered with aluminum foil and was also exposed to sunlight. In between every hour of sunlight exposure, the samples were transferred (in additional 5 minutes) to the foil-covered 24-well plate, and optional markings were made in the wells to enable replenishing with autoclaved water if needed to prevent drying.

Viability test: Following the exposure, three 10 μ L droplets from each sample were pipetted onto a quarter of MPA plates. All plates were then incubated at 20 °C for 16 hours. To check the viability of spray-dried spores coated with sunlight protectants, germination test was conducted in the same manner as described before. The experiment was repeated three times with three replications.

Storability test: Storability test was done in two steps. The first step was to select the right temperature to analyze a potential effect of sunlight protectants on the storability of submerged spores. The second step

Table 1: Spray dryer parameters with range

Parameter	Range
Dry Gas	30 m ³ /hour
Inlet Temperature	65 °C
Spray Gas	900 L/hour
Pump	1.4 mL/minute
Outlet Temperature	48 °C
Product Temperature	35 °C
Unclogging	10 bpm
Filter pressure	49 mbar



Table 2: List of the water-soluble sunlight protectants

Sunlight protectants	Active substances	Manufacturer
Humic Acid Na	Assay of Humic acid ~ 45 - 75%, Loss on drying ~ 25%	Carl Roth GmbH + Co. KG, Karlsruhe, Germany.
Humic Acid K	Potassium – Humates = 80 - 85%, Total Humic Acid = 68 - 73%, Fulvic Acid = 5 - 6%, Potassium (K ₂ O) = 10 - 12%, Dry matter = 83 - 85%, Organic substances = 68 - 73%, pH value = 9.5 - 10.5%, Bulk density = 0.55 - 0.65 Kg/L	Humintech GmbH, Grevenbroich, Germany.
Lignin, Alkali	In powder form, transition temp sintering point 188 °C, density = 1.3 g/mL at 25 °C, surface tension = 43 mN/m (1% aqueous), 5% moisture, 13.4 wt.% loss on heating at 316 °C	Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany.
Skimmed milk	Calorific value 1519 kJ/358 kcal, Fat (thereof saturated fatty acids 1.0 g), 0.6 g Carbohydrates (thereof sugar 51.7 g), Protein 35.5 g, Salt 1.4 g	J. M. Gabler-Saliter Milchwerk GmbH and Co. KG, Obergünzburg, Germany.
Black Tea	Herbal tea, produced by, organic farming from the best growing areas of India, weight = 35g, 20 infusion bags per pack.	Alnatura GmbH, Darmstadt, Germany.
Coffee	Roasted Coffee, finish: ground, coffee type: filter coffee, coffee characteristic: organic, content: 500g, produced in long-term and fair partnerships with farmers in the country of origin, Brazil, Arabica and Robusta beans in organic quality	Alnatura GmbH, Darmstadt, Germany.
Cocoa	95% Cocoa powder, 5% Kaliumcarbonat (E501), Energy content/ calorific value 1620/387 kJ/ kcal, Fat 21 g = thereof saturated fatty acids 13 g, Carbohydrates 12 g (thereof sugar 0.5 g) Protein 0.05 g, Dietary fiber 22 g, Salt 0.04 g (thereof starch 31 g). From Africa, middle and south America.	Alnatura GmbH, Darmstadt, Germany.
Calcofluor white	Calcofluor white M2R, 1 g/L, Evans blue, 0.5 g/L, λ_{max} = 423 - 443 nm, form: liquid	Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany.
Green Tea	Comes from organic farming	Alnatura GmbH, Darmstadt, Germany.



was to store the formulated spores for different duration at previously selected temperature.

Storage temperature: Freshly produced spray-dried spores were kept at 20 °C and 6 °C for 1 week to get first information of a potential influence of storage temperature on the viability of spores. Spray-dried spores were stored in a sterile 15-mL Eppendorf conical test tubes (Carl Roth GmbH + Co. KG., Karlsruhe, Germany). The experiment was repeated three times.

Storage weeks: For long-term storage test, spray-dried spores were kept for 1, 2, 4, 8 and 12 weeks at 6 °C. After that, germination test was done by the same manner after the respective weeks. Sample of spray-dried spores were taken from the same conical test tube for the following weeks of storage. The experiment was conducted with three repetitions and three replications.

STATISTICAL ANALYSIS

For screening water-soluble sunlight protectants, data analysis in R Studio (Version 1.4.1106) (RStudio Team 2020) showed a clear correlation between wavelength and optical density. Data are presented as mean ± SD or adjusted mean ± SE, based on the model used. To ensure ANOVA assumptions, residuals were visually inspected; normality was checked via QQ-Plot and variance homogeneity via Residuals-Prediction-Plot. Model selection was guided by Akaike Information Criterion (AIC) [18] through backward elimination. Post hoc comparisons were conducted with Tukey HSD ($\alpha = 0.05$) using the emmeans package (Lenth 2023). For estimating germination test for feasibility, viability and storability test, following LM has been executed:

LM ($y \sim \text{media} + \text{hour} + \text{media}:\text{hour} + \text{repetition} + \text{replication}$)

For Kaplan-Meier analysis, survival curves over time were created with the survival R package (Therneau 2023) with survfit function and compared with survival curves using the log-rank test (survdiff function) to determine global effects.

RESULTS

Feasibility test: First, a feasibility test was performed to proof whether freshly produced submerged spores from liquid culture can

survive the spray-drying process. It was found that spray-drying has a direct influence on the viability (ANOVA; $F = 8.49$, $df = 1$, $p < 0.01$). After pairwise comparison (Figure 1) germination of freshly produced spores (99.25%) was significantly higher ($df = 8$, $p < 0.01$) than of spray-dried spores (95.6%). Though spray-dried spores showed significantly lower germination than freshly produced spores, for both the germination rate was higher than 95%. Therefore, it can be said that spray-drying is feasible and this formulation technique can be used for further experiments.

Screening of water-soluble sunlight protectants: While screening the water-soluble sunlight protectants, it has been observed that all the samples (Humic acid Na, humic acid K, lignin, green tea, cocoa, calcofluor white, black tea, coffee, skimmed milk) were showing there absorbance > 0 OD600 throughout the whole wavelength range (280 - 800 nm) but with different curve characteristics (Supplementary Figure 1).

Viability test: Following feasibility testing and absorption measurements, submerged spores coated with six sunlight protectants were analyzed for viability under 1–4 hours of simulated sunlight. Humic acid Na, humic acid K, and lignin were removed due to product adherence issues during spray-drying, and cocoa was excluded due to visibility issues on MPA plates. Kaplan-Meier survival curves (Figure 2) showed significant differences in germination rates across formulations, with black tea outperforming others (log-rank test, $p < 0.0001$). Black tea-coated spores displayed significantly higher germination probabilities than control ($p < 0.0001$), skimmed milk ($p < 0.001$), coffee ($p < 0.0001$), calcofluor white ($p < 0.001$), and green tea ($p < 0.0001$).

Further analysis indicated that formulation type significantly impacted germination under sunlight (ANOVA; $df = 5$, $F = 37.40$, $p < 0.001$), as did exposure duration ($df = 4$, $F = 180.71$, $p < 0.001$). Without sunlight (Figure 3), germination rates for black tea (94.8%) and calcofluor white (88.8%) did not significantly differ from the control, whereas coffee (75.2%) and green tea (56.2%) were lower ($df = 325$, $p < 0.0001$). After two hours, black tea-coated spores maintained higher germination (75.6%, $df = 325$, $p < 0.0001$). After three hours, black tea (66.8%, $df = 325$, $p < 0.0001$) and calcofluor white (47.8%, $df = 325$, $p < 0.0001$) outperformed the control. After four hours, black tea provided the highest germination (48.3%), significantly surpassing skimmed milk (17.8%, $df = 325$, $p < 0.0001$).

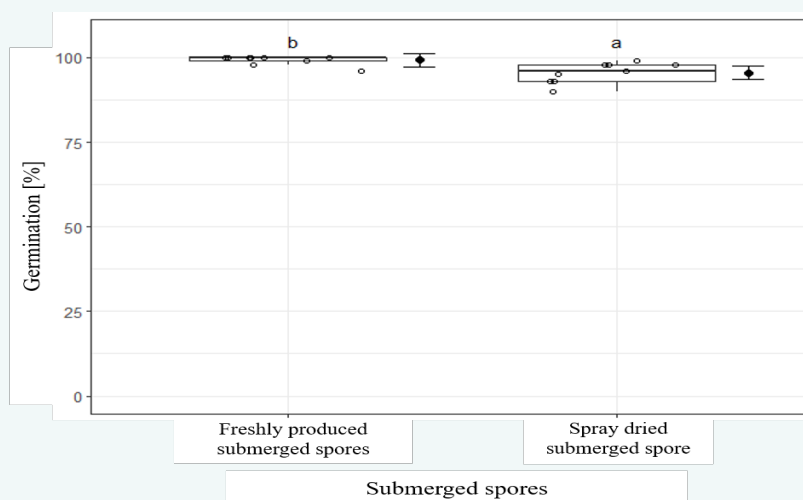


Figure 1: Germination rate of submerged spores of JKI-BI-1496 before and after spray-drying. The germination rate was analyzed after 16 hours of incubation on MPA at 20 °C. Jittered boxplots consist of the median and the 25% and 75% quantile. Black dots and error bars represent adjusted mean with 95% confidence limits. Means with the same letters are not significantly different (Tukey HSD test, $\alpha = 0.05$, $n = 3 \times 3$).

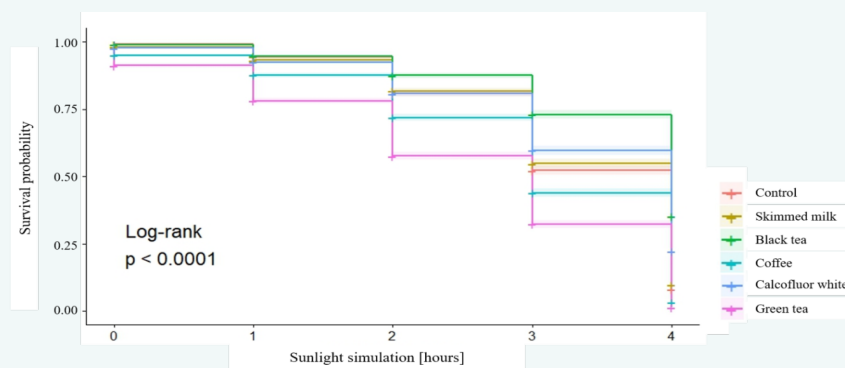


Figure 2: Overall survival probability (Kaplan-Meier analysis) of the spray-dried submerged spores [% germination] coated with different sunlight protectants (control, skimmed milk, black tea, coffee, calcofluor white and green tea) over 4 hours simulated sunlight exposure. The p-value (log-rank test, $\alpha = 0.05$, $n = 3 \times 3$) indicates differences among the germination rate of submerged spores formulated with sunlight protectants within the respective sunlight simulation time.

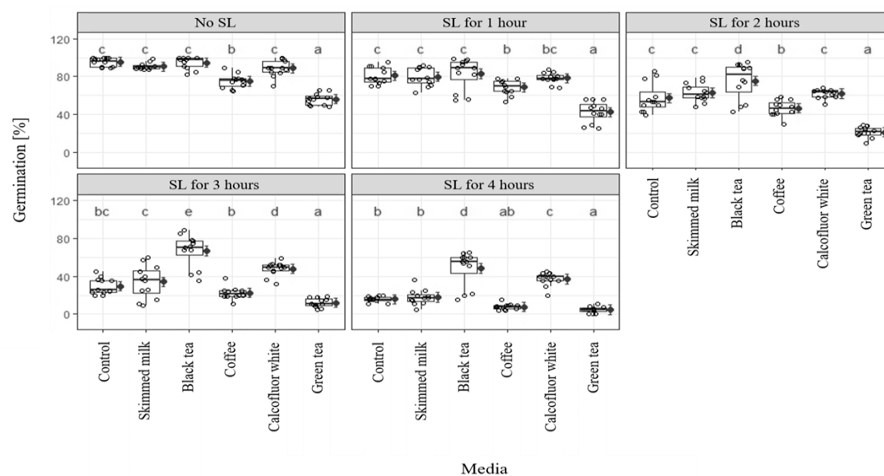


Figure 3: Germination [%] of spray-dried submerged spores of *C. fumosorosea* coated with different sunlight protectants (control, skimmed milk, black tea, coffee, calcofluor white and green tea) under simulated sunlight (for 0, 1, 2, 3 and 4 hours). The germination was analysed after 16 hours of incubation on MPA at 20 °C. Jittered boxplots consist of the median and the 25% and 75% quantile. Black dots and error bars represent adjusted mean with 95% confidence limits. Means with the same letters are not significantly different (Tukey HSD test, $\alpha = 0.05$, $n = 3 \times 3$) (SL = Sunlight simulation).

= 325, $p < 0.0001$), coffee (7.75%, $df = 325$, $p < 0.0001$), calcofluor white (37.1%, $df = 325$, $p = 0.02$), green tea (4.67%, $df = 325$, $p < 0.0001$), and the control (15.8%, $df = 325$, $p < 0.0001$). Survival analysis revealed that black tea extended spore viability under simulated sunlight to 3.92 hours for 50% germination, compared to calcofluor white (2.98 hours), skimmed milk (2.42 hours), and control (2.33 hours).

STORABILITY TEST:

Storage temperature: After storing the spray-dried submerged spores at 6 °C and 20 °C for 1 week, the result reveals that temperature significantly influenced germination rates (ANOVA; $df = 1$, $F = 237.53$, $p < 0.001$). When pairwise Tukey test was done (Figure 4), it was seen that at 20 °C, none of the different formulated spray-dried spores germinated more than 10.3%. On other hand, at 6 °C, spores formulated with different sunlight protectants germinated between over 50% and 90% after 1 week of storage. Therefore, for further storage experiment, 6 °C was taken as standard temperature.

Storage over time: The Kaplan-Meier survival curves (Figure

5) indicated distinct germination rates across formulations, with a significant difference among groups (log-rank test, $p < 0.0001$). ANOVA showed that both formulation and sunlight protectant type significantly influenced germination ($df = 5$, $F = 40.48$, $p < 0.001$), as did storage time ($df = 5$, $F = 108.71$, $p < 0.001$). Initially, skimmed milk (96.7%) and black tea (96.1%) coatings maintained germination rates close to the control (97.2%), while calcofluor white (71.8%), coffee (57.1%), and green tea (58.4%) coatings showed lower rates ($df = 284$, $p < 0.0001$). Over time (Figure 6), skimmed milk and black tea retained higher germination rates, especially in the first and second weeks. By the eighth week, black tea-coated spores (72.4%, $df = 284$, $p = 0.0002$) and skimmed milk-coated spores (65.8%, $df = 284$, $p = 0.052$) still performed well compared to the control, while coffee (19.0%, $df = 284$, $p < 0.0001$) and green tea (12.1%, $df = 284$, $p < 0.0001$) saw significant declines. At twelve weeks, calcofluor white (44.89%) outperformed most other protectants except black tea ($df = 284$, $p < 0.0001$). The time to a 50% reduction in germination varied by treatment: skimmed milk and black tea provided extended viability at 8.32 and 9.13 weeks, respectively, while coffee-coated spores declined rapidly at 0.44 weeks. Calcofluor white delayed the reduction to 9.92

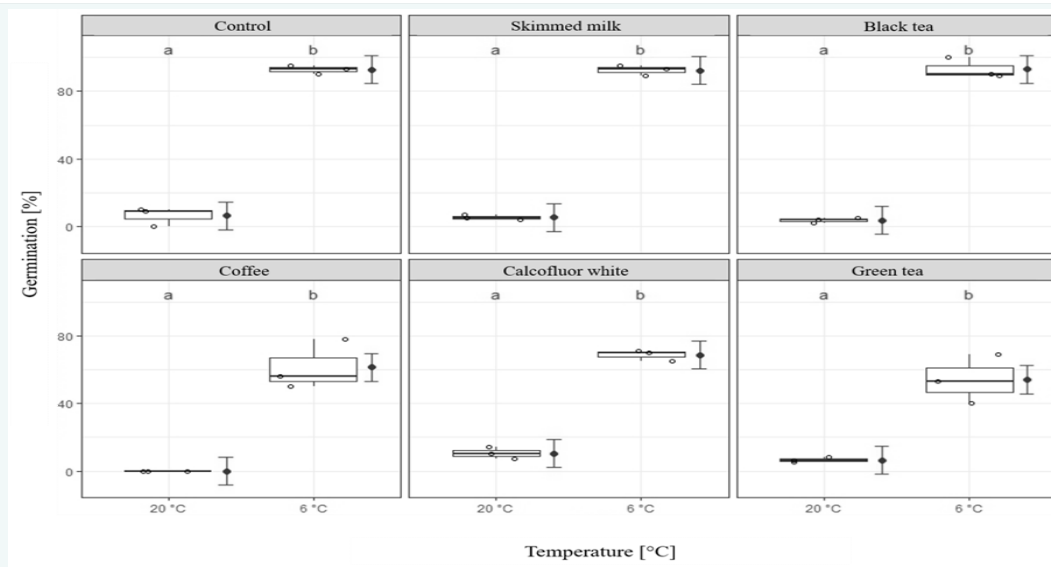


Figure 4: Influence of two different storage temperatures on germination [%] of submerged spores of *C. fumosorosea* coated with five potential sunlight protectants. Duration of the storage was 1 week. The germination was analysed after 16 hours of incubation on MPA at 20 °C. Jittered boxplots consist of the median and the 25% and 75% quantile. Black dots and error bars represent adjusted mean with 95 % confidence limits. Means with the same letters are not significantly different. (Tukey HSD test, $\alpha = 0.05$, $n = 3$).

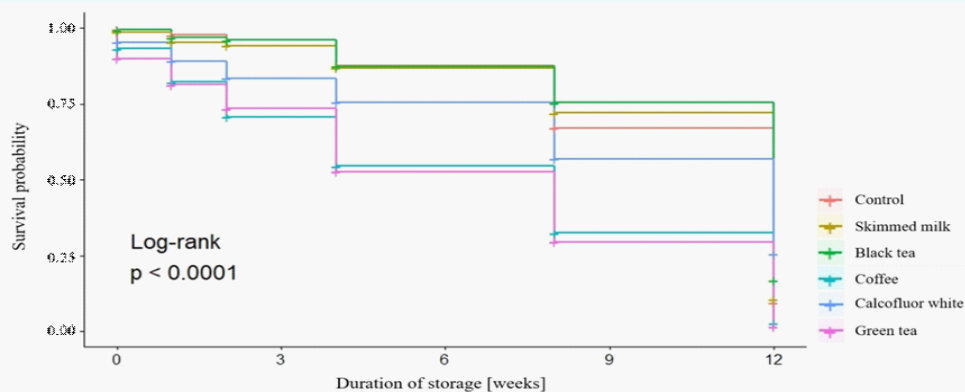


Figure 5: Overall survival probability (Kaplan-Meier analysis) of the spray-dried submerged spores [% germination] coated with sunlight protectants (control, skimmed milk, black tea, coffee, calcofluor white and green tea) after storage of 1, 2, 4, 8 and 12 weeks. The statistic of shown p-values (log-rank test, $\alpha = 0.05$, $n = 3 \times 3$) and indicates differences among the germination rate of submerged spores coated with different sunlight protectants for respective storage time.

weeks, and green tea reached 50% at 1.40 weeks.

DISCUSSION

This study aimed to optimize the spray-drying process for *C. fumosorosea* submerged spores, ensuring viability and long-term storage with sunlight protectants. Earlier research identified nutritional and environmental factors for rapid production of desiccation-tolerant submerged spores [19,20]. Effective drying of submerged spores, vital for microbial biocontrol, is achievable through solid or oil-based formulations; however, solid forms are cost-effective and straightforward [21,22]. Spray-drying was selected for this study due to its scalability, cost-effectiveness, and ability to rapidly process large volumes at relatively low temperatures—features that make it suitable for industrial-scale formulation of biocontrol agents. While techniques like freeze-drying and electrospinning also offer preservation and encapsulation advantages, spray-drying provides a practical balance between processing efficiency and spore viability, particularly when optimized for heat-sensitive

biological materials [10-23]. Submerged spores, however, are vulnerable to desiccation, necessitating the addition of protective agents like skimmed milk, known for membrane-stabilizing and sunlight-protectant properties. Using 5% skimmed milk in this study yielded over 90% germination in JKI-BI-1496 spores, consistent with other studies [10-24].

After confirming the feasibility of spray-dried submerged spores, the next phase involved screening water-soluble sunlight protectants. While oil-based protectants are commonly used [5-24], water-soluble options offer unique advantages for *C. fumosorosea*, such as dual protection by absorbing, blocking, or reflecting UV radiation [25]. In this study, all nine tested protectants demonstrated sunlight absorbance ($OD > 0$), indicating effective sunlight protection. These compounds have also been validated in various studies for their protective efficacy [26-28].

To protect submerged spores from sunlight, incorporating sunlight protectants into fungal formulations is effective, though results have varied in lab studies [5-7]. In this study, unprotected submerged spores

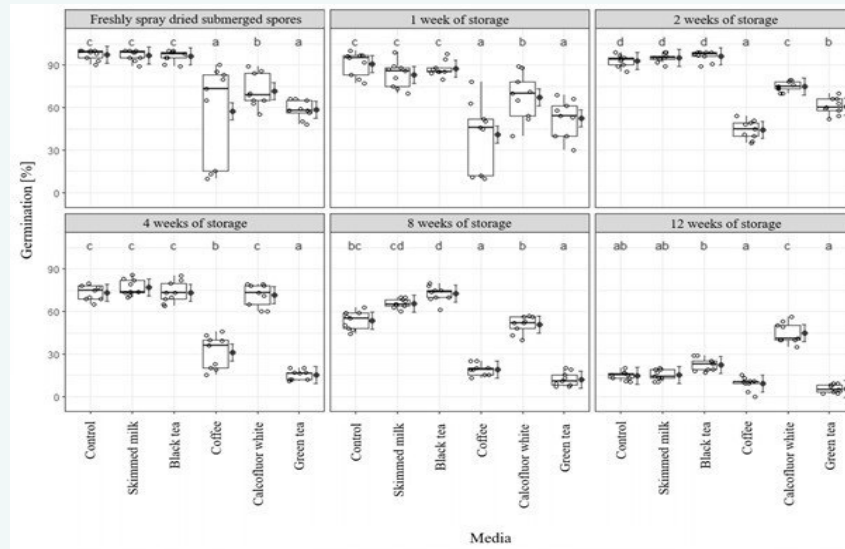


Figure 6: Germination [%] of spray-dried submerged spores of *C. fumosorosea* coated with different sunlight protectants for storage after freshly produced spores, 1 week, 2 weeks, 4 weeks, 8 weeks, and 12 weeks of storage. The germination was analysed after 16 hours of incubation on MPA at 20 °C. Jittered boxplots consist of the median and the 25% and 75% quantile. Black dots and error bars represent adjusted mean with 95% confidence limits. Means with the same letters are not significantly different (Tukey HSD test, $\alpha = 0.05$, $n = 3 \times 3$).

showed reduced germination after two hours of sunlight simulation, consistent with findings on *Metarhizium spp.* [8-28]. However, black tea-coated spores exhibited the highest germination rates (over 50%) after four hours of sunlight, outperforming other formulations. Black tea's UV protection is attributed to its high phenolic content, including theaflavins, thearubigins (75-82% of total phenolics), epicatechin gallate, and caffeine, which provides antioxidant and UV-absorbing properties [29-31]. These findings align with previous research, supporting black tea's efficacy as a UV protector, likely due to these phenolic compounds and caffeine [28-32].

After confirming solar protection, the effect of sunlight protectants on storability was assessed. In this study, formulated submerged spores stored at 6 °C retained over 50% germination, while those at 20°C showed less than 10.3% germination. This aligns with research showing lower temperatures enhance long-term spore viability. For instance, [33] found that spray-dried *Metarhizium* spores maintained over 70% viability at 5°C for four years but deteriorated at 30 °C. Similarly, *B. bassiana* conidia retained 80% viability at 4°C for six months [34]. Studies with *C. fumosorosea* and *B. bassiana* also report high survival under refrigeration (Jackson et al. 2006) [23], and modified atmosphere storage at 4°C improved *Metarhizium spp.* blastospore half-life by 2.1 times [13].

Storage experiments showed that black tea-coated spores maintained the highest germination up to 8 weeks, but by 12 weeks, calcofluor white-coated spores surpassed others, retaining over 50% germination. Calcofluor white, a known UV protectant and adjuvant for EPF, provides UV protection at 1–10 g/L without inhibiting *B. bassiana* growth [6-35]. In this study, calcofluor white-coated spores demonstrated significantly higher germination under simulated sunlight, second only to black tea.

Reddy et al. [36], developed *B. bassiana* tablets with calcofluor white, achieving an 18-month shelf life at 4–8 °C. Though studies on *C. fumosorosea* shelf life with calcofluor white are limited, its extensive use in fungal staining and microbiology is well-documented [37]. Calcofluor white's composition includes nitrogenous elements that bind to cellulose and chitin, potentially inhibiting fungal growth and acting as a preservative [38]. Its stability further supports prolonged shelf life,

making it an effective sunlight protectant for *C. fumosorosea* spores [37-48].

CONCLUSION

In conclusion, it can be said that spray-drying has been shown to be an effective preservation method, achieving a 90% germination of submerged spores of JKI-BI-1496 *C. fumosorosea*. The addition of black tea, a natural source of polyphenols and antioxidants, further reinforces this approach as a shield against solar radiation. Black tea has properties that can protect against sunlight-induced degradation, which can help to extend the longevity of treated materials. Additionally, the inclusion of calcofluor white, which is known for its preservative qualities and moderate sunlight protection capabilities, can help to further extend the shelf life of treated substances while maintaining their efficacy over prolonged periods. This approach provides a comprehensive solution for ensuring material integrity and performance under varying environmental conditions, highlighting the reliability of the preservation process and its practical applicability in a variety of academic and industrial settings.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper



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