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INFLUENCE OF UV-B RADIATION ON INITIAL DEVELOPMENT AND CONTROL OF PATHOGENS IN SOYBEAN SEEDS

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ABSTRACT

Ultraviolet-B (UV-B) irradiation is being explored as a non-chemical approach for seed sanitation with potential effects on early vigor. In soybean (*Glycine max* (L.) Merr.), preserving both physiological and sanitary seed quality is critical for stand establishment. We evaluated UV-B at a fixed irradiance (1.5 W m^{-2}) across five durations (0, 10, 20, 30, 40 min), delivering cumulative doses of 0.9, 1.8, 2.7, and 3.6 kJ m^{-2} (0.009, 0.018, 0.027, 0.036 J cm^{-2}). Endpoints included viability (germination, dead seeds), vigor (first count, dry mass), seedling performance (shoot and root length, fresh mass), and sanitary status (blotter test). Responses were dose-dependent with hormetic features: prolonged exposures (30-40 min) markedly reduced germination (79% in the control vs. 44% and 28%), and the observed increases in shoot and root length at 30 min occurred only among surviving seedlings, providing no agronomic advantage due to the concomitant loss of viability. Total pathogen incidence (seeds with ≥1 detectable pathogen) decreased from 98% (control) to 55% at 40 min, with the largest reductions in *Rhizopus* spp. and visible bacterial colonies. Given the trade-off between sanitation and viability, 10–20 min (0.9–1.8 kJ m^{-2}) emerges as a practical operational window that maintains acceptable germination while beginning to reduce pathogen load. Because blotter tests cannot resolve bacterial identity, bacterial results are reported as visible colonies only. UV-B should be considered a complementary, sustainable tool within integrated seed-borne disease management rather than a stand-alone replacement.

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INTRODUCTION

In Paraguay, soybean (*Glycine max* L.) is a strategic crop, with an estimated production of 10.1 million tons in the 2023/24 season, contributing substantially to the national GDP (CAPECO, 2025). Beyond physiological vigor and viability, seed sanitary status is decisive for stand establishment and field performance, guiding decisions in seed health programs (Scariot *et al*., 2017; Nóbrega & do Nascimento, 2020; Rey *et al*., 2009). Among seed-associated fungi of major economic relevance in soybean are *Sclerotinia sclerotiorum* (white mold), *Rhizoctonia solani* (damping-off and root rot), *Aspergillus* spp. (including aflatoxin-producing *A. flavus* linked to storage), and *Rhizopus* spp., frequently detected as post-harvest/storage contaminants (Goulart, 2018; Anwar *et al*., 2013). These pathogens depress vigor and germination and can introduce primary inoculum into soil, favoring early-season epidemics (Goulart, 2018; Anwar *et al*., 2013; Nóbrega & do Nascimento, 2020).

Ultraviolet-B (UV-B) irradiation has emerged as a non-chemical seed-sanitation approach with potential ancillary effects on seedling performance. While UV-B can be phytotoxic depending on dose (irradiance × exposure time), controlled exposures may induce defense

responses and reduce pathogen incidence (Winter & Rostas, 2008; Foroughbakhch Pournavab *et al*., 2019; Loconsole & Santamaria, 2021). Mechanistically, UV-B can directly damage microbial propagules and nucleic acids and also modulate plant defense signaling—e.g., activating phenylpropanoid pathways and phytoalexins—thereby enhancing disease resistance (Rastogi *et al*., 2013; Vanhaelewyn *et al*., 2016; Meyer *et al*., 2021). Beyond direct photolesions, plant perception of UV-B is mediated by the UV RESISTANCE LOCUS 8 (UVR8) photoreceptor, which triggers downstream signaling and cross-talk with phytohormones and the phenylpropanoid pathway, culminating in the accumulation of phytoalexins and pathogenesis-related proteins under calibrated exposures (Jenkins, 2009; Vanhaelewyn *et al*., 2016; Meyer *et al*., 2021). Empirical evidence shows dose- and species-dependent effects of UV radiation on germination and early growth across crops, including soybean and common bean, and selective inactivation/suppression of seed-associated fungi such as *Aspergillus* and *Rhizoctonia* (Stefanello *et al*., 2023; Hernandez-Aguilar *et al*., 2021; Al-Gabr *et al*., 2013; Foroughbakhch Pournavab *et al*., 2019). Despite these advances, seed-focused UV-B studies in soybean remain limited, particularly under tropical/subtropical conditions relevant to Paraguay (Foroughbakhch Pournavab *et al*., 2019; Loconsole & Santamaria, 2021). Moreover, the phytopathological implications i.e., balancing sanitary gains against potential losses in germination are not fully resolved (Winter & Rostas, 2008; Meyer *et al*., 2021). Here, we evaluate graded UV-B doses (operationalized as exposure time at fixed irradiance) on (i) seed physiological quality and (ii) total pathogen incidence, defined as the percentage of seeds carrying ≥1 detectable pathogen in a standardized blotter test (Goulart, 2018). We test the hypothesis that there exists a practical exposure window that delivers meaningful sanitary control while preserving acceptable germination, positioning UV-B as a complementary tool within integrated seed disease management (Scariot *et al*., 2017; Nóbrega & do Nascimento, 2020; Rey *et al*., 2009).

MATERIAL AND METHODS

Experimental Site and Plant Material

The study was conducted at the Seed Laboratory, Faculty of Agricultural Engineering, Universidad Nacional del Este (UNE), Alto Paraná, Paraguay, between September and December 2024. Commercial soybean (*Glycine max* L.) seeds, cultivar CM 422 "Milagrosa," were used. A total of 1,000 seeds were evaluated across five UV-B exposure times (0, 10, 20, 30, 40 min) and four replicates. Each replicate comprised 50 seeds and constituted the experimental unit, following ISTA (2017) rules.

UV-B Radiation Treatment

A completely randomized design with five exposure durations and four replicates was used. Control seeds (0 min) received no irradiation, and each treatment was applied independently. UV-B radiation was supplied by a compact lamp (nominal 15 W) emitting in the 280–320 nm range. Irradiance at seed level was held at 1.5 W m^{-2} (0.15 mW cm^{-2}), verified with a digital radiometer. The lamp was warmed up for 5 min prior to each run to stabilize output. Seeds were placed in Gerbox™ containers at 15 cm from the source and spaced to avoid overlap. The cumulative UV-B dose was calculated as H (J m^{-2}) = E (W m^{-2}) × t (s), with E = 1.5 W m^{-2} and exposure times of 10, 20, 30, and 40 min, yielding 0.9, 1.8, 2.7, and 3.6 kJ m^{-2} (i.e., 0.009, 0.018, 0.027, 0.036 J cm^{-2}).

Physiological Quality Assessment

Immediately after irradiation, seeds were placed on Germitest® paper, moistened with distilled water to 2.5× paper dry mass, and rolled (50 seeds per roll; 4 rolls per treatment, totaling 200 seeds per treatment). Rolls were incubated at 25 ± 2 °C in a germination chamber (ISTA, 2017).

The variables evaluated were:

a) First Germination Count (FGC): percentage of normal seedlings at 5 days after sowing (DAS), scored per ISTA (2017) criteria.
b) Germination Percentage (G%): Final count performed at 8 days after sowing (ISTA, 2017), expressed as the percentage of normal seedlings.
c) Dead Seeds Percentage (DS%): seeds failing to initiate germination or producing non-viable tissues at the final count.
d) Seedling length: shoot length (SL) and root length (RL) measured at 8 DAS on 10 normal seedlings per replicate, sampled sequentially left-to-right to avoid selection bias (mm ruler; reported in cm).
e) Fresh and dry mass: at 8 DAS, 10 normal seedlings per replicate. Fresh mass was weighed immediately. For dry mass, shoots and roots (including cotyledons) were dried at 65 °C (forced-air oven) to constant weight (~72 h). Masses were recorded on a precision balance.

Normal/abnormal seedling definitions followed ISTA (2017).

Sanitary quality assessment

Seed health was assessed using the Blotter-test method (Brasil, 2009). For each treatment, 200 seeds (4 × 50) were pre-chilled at −20 °C for 24 h to suppress germination and favor fungal expression, then incubated for 7 days at 25 ± 2 °C under a 12 h photoperiod (fluorescent light) in a BOD chamber. Three variables were recorded: (i) pathogen-free seeds (%), the proportion of seeds without detectable microbial growth; (ii) total pathogen incidence (%), the proportion of seeds carrying ≥1 detectable pathogen (note: genera can co-occur on the same seed, thus per-genus incidences do not sum to total incidence); and (iii) incidence by genus (%) and colony counts for *Aspergillus* spp., *Rhizopus* sp., *Rhizoctonia* sp., *Sclerotinia* sp., and visible bacterial colonies.

Fungal characterization followed macroscopic and microscopic morphology (bright-field optical microscopy, ~400×), recording conidiophores, conidia, sporangia, mycelial features, and sclerotia. Identification was at genus level for routine screening; presumptive species were assigned only when diagnostic structures were present (e.g., *Sclerotinia sclerotiorum* based on sclerotia; *Rhizoctonia solani* based on right-angle branching and septation). Bacterial presence was recorded as visible colonies on blotter arising from individual seeds; this approach supports detection but does not provide reliable genus- or species-level identification. Therefore, bacterial data are reported descriptively as "bacteria (visible colonies)," and no taxonomic inferences are made. Future work should employ selective media, biochemical tests, and/or molecular methods for resolution.

Statistical analysis

Data were analyzed in a completely randomized design with treatment (exposure time) as the fixed factor and replicate as the experimental unit. Proportions (first germination count, seed germination percentage, dead seeds percentage, pathogen-free seeds, and total incidence) were transformed ($\sqrt{x}/100$) to stabilize variance before analysis of variance (ANOVA). Means were compared by Tukey's test ($\alpha = 0.05$). Dose-response trends were evaluated by linear and quadratic regressions on transformed data. Results in tables/figures are shown as back-transformed means with Tukey group letters. F-values, degrees of freedom, and p-values are provided in Supplementary Material (Tables S1-S7). Analyses were performed in SAS 15.3 (SAS Institute Inc., Cary, 2023).

RESULTS

The first germination count (FGC) (Figure 1A) and seedling dry weight (SDW) (Figure 1B) showed no significant treatment effects (ANOVA, $p > 0.05$), indicating that the UV-B doses tested did not compromise initial vigor or dry biomass accumulation. FGC ranged from 51% (0 min) to 55% (10 min), suggesting a non-significant low-dose stimulus. SDW tended to be higher in the control and at 30 min but did not differ by Tukey's test ($\alpha = 0.05$).

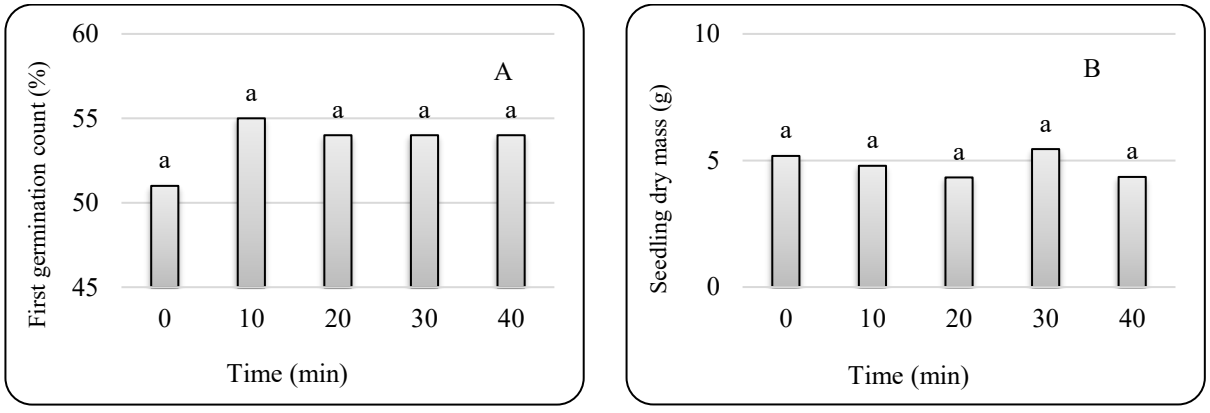


Figure 1. Effect of UV-B radiation exposure on soybean seeds regarding first count of germination (%) (A) and seedling dry weight (g) (B) at different exposure times. Means (n = 4); different letters indicate Tukey's test ($\alpha = 0.05$).

For the seed germination percentage (G%) (Table 1; Figure 2), the response was non-linear. Germination rose numerically at 10 min (87%) versus the control (79%) but without statistical separation and then declined sharply for longer exposures: 44% at 30 min and 28% at 40 min (ANOVA, $F_{4,15} = 29.58$, $p < 0.0001$ see Table S1). A

simple linear fit captured the overall downward trend with increasing exposure time ($R^2 = 0.68$). The dead seeds (%) differed among treatments (ANOVA, $F_{4,15} = 5.93$, $p = 0.0046$ see Table S2); 10 min had the lowest mean (9%), while 0, 20, 30 and 40 min formed a higher, statistically similar group (Table 1).

Table 1. Seed germination percentage (%) and dead seeds percentage (%) of soybean seeds exposed to UV-B (0, 10, 20, 30, 40 min).

Treatment (min)	Germination (%)	Dead Seeds (%)
0 (Control)	79 a	20 a
10	87 a	9 b
20	80 a	20 a
30	44 b	17 a
40	28 b	18 a

Means (n = 4); different letters indicate Tukey's test (α = 0.05).

Exposure significantly affected seedling growth. Shoot and root length peaked at 30 min (both p < 0.0001; see Tables S3–S4). However, this elongation reflects selective growth among surviving seedlings after a 54% reduction in germination relative to the control (Figure 2). Thus, these physiological responses do not translate into a net agronomic benefit at the population level.

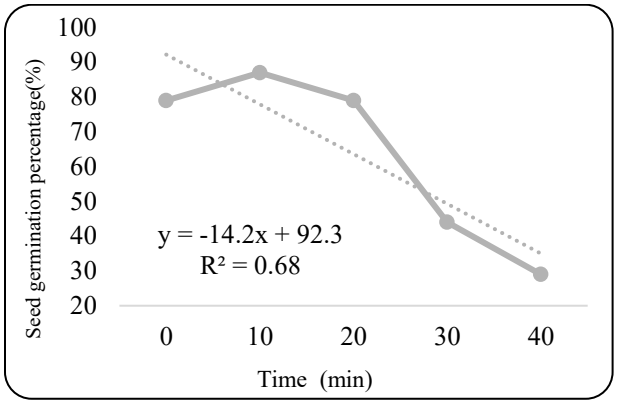


Figure 2. Seed germination (%) of soybean (*Glycine max* (L.) Merr.) after UV-B exposure at 1.5 W m^{-2} for 0, 10, 20, 30, or 40 min. Points show means (n = 4); different letters indicate Tukey's HSD (α = 0.05). A linear regression line summarizes the overall decreasing trend with time ($R^2 = 0.68$).

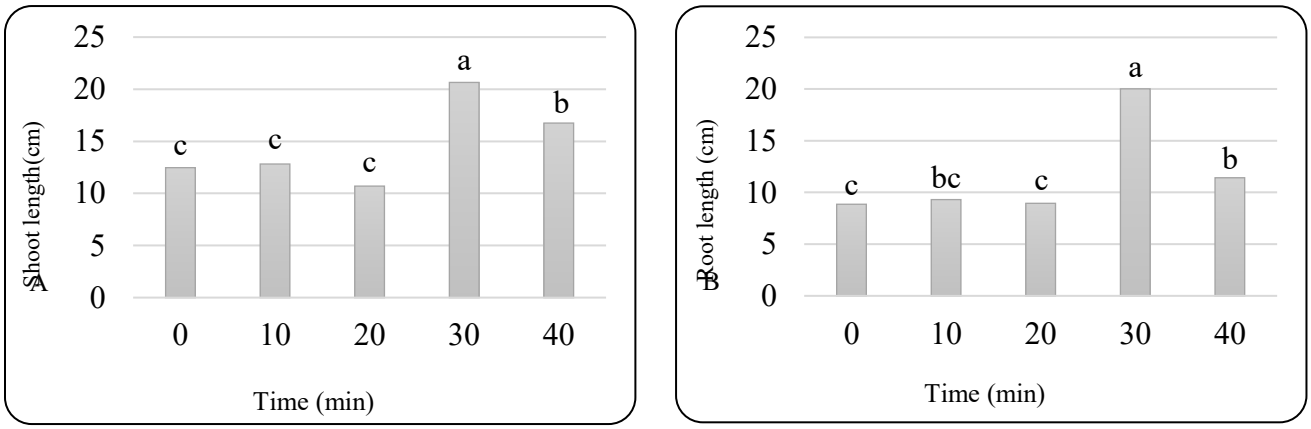


Figure 3. Shoot length (A) and root length (B) of seedlings as a function of exposure time (0, 10, 20, 30, and 40 minutes). Means (n = 4); different letters indicate Tukey's test (α = 0.05).

Regarding the variables Seedling Fresh Mass (FM) (Figure 4A) and Seedling Dry Mass (DM) (Figure 4B), significant differences were observed only in Fresh Mass (Figure 4A) (ANOVA; $F_{(}4,15_{)} = 14,73$; p < 0,0001, see Table S5). The 10-minute radiation exposure was statistically different from the other treatments, presenting significantly lower values in Fresh Mass content (Figure 4A). This indicates that the 10-minute exposure significantly reduced the Fresh Mass, while the other times (0, 20, 30, and 40 min) maintained values similar to the control.

Conversely, for the Seedling Dry Mass variable (Figure 4B), no significant differences were observed among the UV-B light exposure times. Therefore, it can be affirmed that UV-B radiation, under the times evaluated, did not consistently affect the plant's dry mass, but may reduce the fresh mass when applied for 10 minutes.

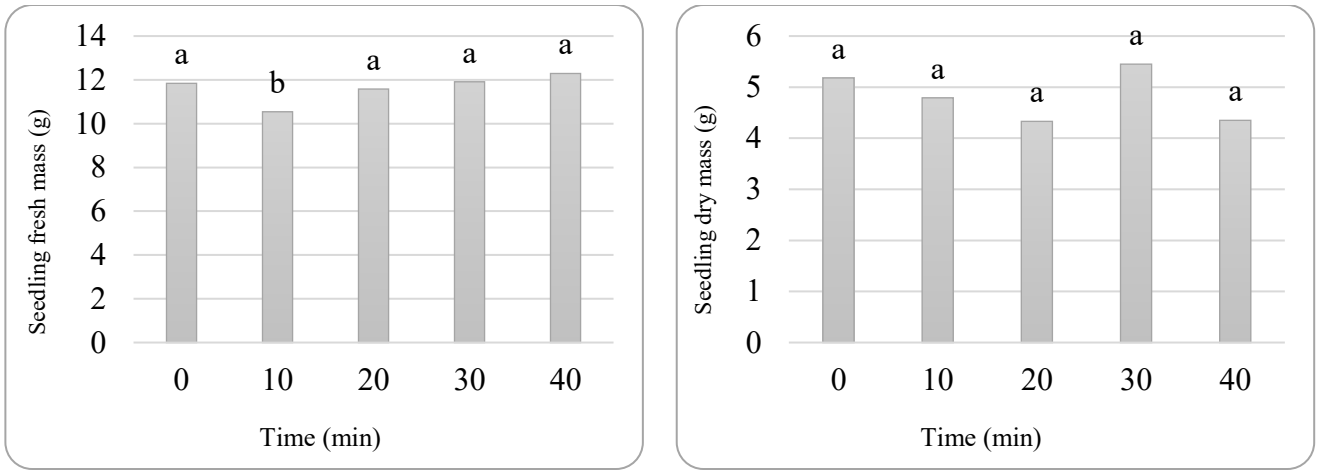


Figure 4. Seedling fresh mass (A) and seedling dry mass (B) of soybean seedlings exposed to UV-B radiation. Means (n = 4); different letters indicate Tukey's test (α = 0.05).

For seed health, we defined: (i) pathogen-free seeds (%) = proportion of seeds without detectable growth; (ii) total pathogen incidence (%) = proportion of seeds with ≥1 detectable pathogen; and (iii) incidence by genus (%) and colony counts. UV-B significantly increased pathogen-free seeds and reduced total pathogen incidence (ANOVA for pathogen-free seeds, $F_{4,15} = 41.60$, $p < 0.0001$ see Table S6). Pathogen-free seeds rose from 2% (0 min) to 45% (40 min), while total incidence fell from 98% to 55% (Figure 5). The most consistent reductions occurred at 30–40 min.

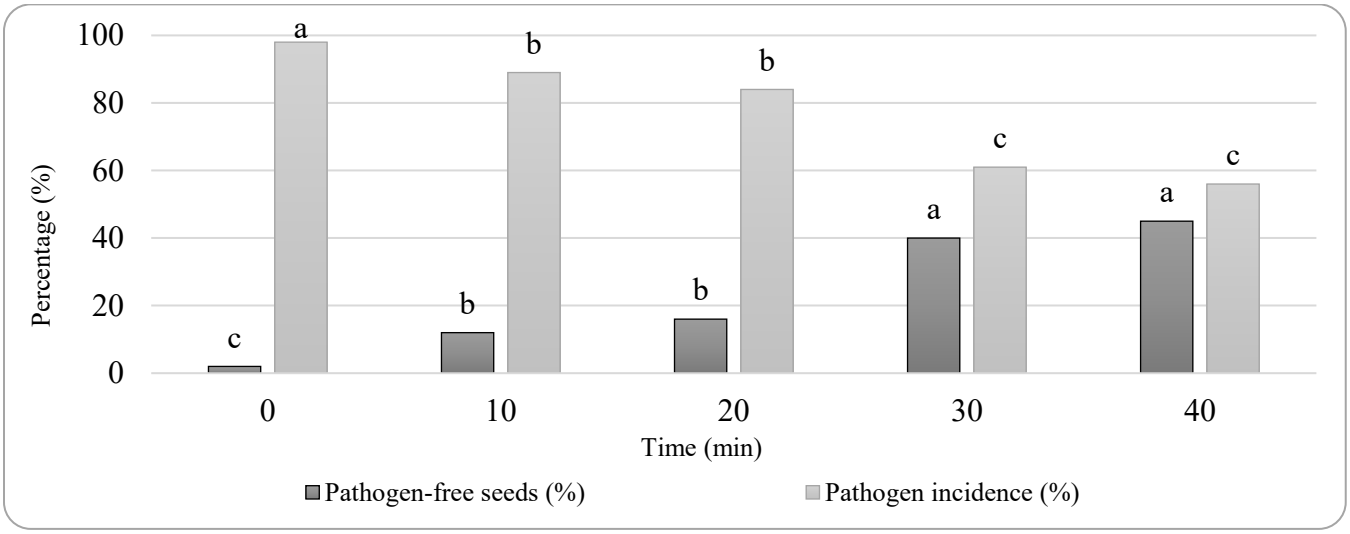


Figure 5. Pathogen-free seeds (%) and total pathogen incidence (%) in soybean (*Glycine max* (L.) Merr.) after UV-B exposure at 1.5 W m^{-2} for 0, 10, 20, 30, or 40 min. Values are means (n = 4); different letters within each response variable indicate Tukey's HSD (α = 0.05). "Total incidence" denotes the percentage of seeds with ≥ 1 detectable pathogen by blotter test; per-genus incidences do not sum to this total because multiple genera can co-occur on a single seed.

Five microbial categories were detected: *Aspergillus spp.*, *Sclerotinia sp.*, *Rhizoctonia sp.*, *Rhizopus sp.*, and bacteria (Figures 6 and 7).

Only *Sclerotinia sp.* colony counts differ significantly across times (Figure 6B) (ANOVA; $F_{(4,15)} = 3,65$; $p < 0,0287$, see Table S7). They were highest at 10 min (7 colonies per 50 seeds) and lowest at 30 min (1 colony), suggesting colonization intensity is time-dependent for this pathogen. Other genera and bacteria varied numerically but without significant differences in colony counts.

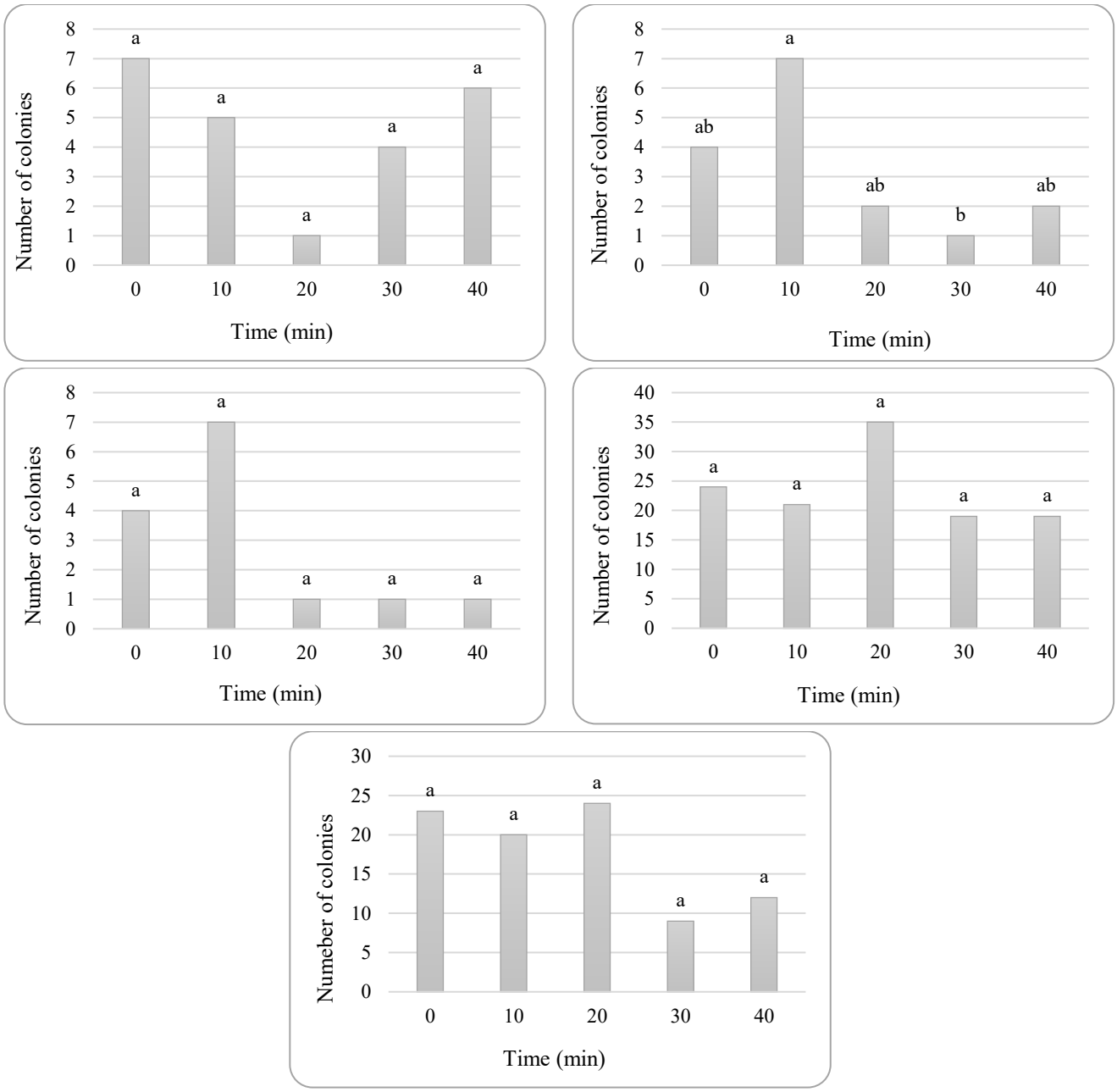


Figure 6. Number of colonies, including *Aspergillus spp. (*A), *Sclerotinia sp. (*B), *Rhizoctonia sp. (*C), *Rhizopus sp. (*D) and bacteria *(*E), in soybean seeds exposed to UV-B radiation. Means (n = 4); different letters indicate Tukey's test (α = 0.05).

The incidence heatmap (Figure 7) revealed selective UV-B effects. At 0 min, *Rhizopus* sp. (22.5%) and bacteria (23.8%) predominated; at 30 min, *Rhizopus* sp. dropped to 8.5% and bacteria to 19.0% (both < control), with additional decreases or stability at 40 min.

UV-B imposed a clear trade-off: strongest sanitary gains at 30–40 min coincided with major germination losses. Shorter exposures (10–20 min) provided a better balance between germination, dead seeds, and microbial reduction.

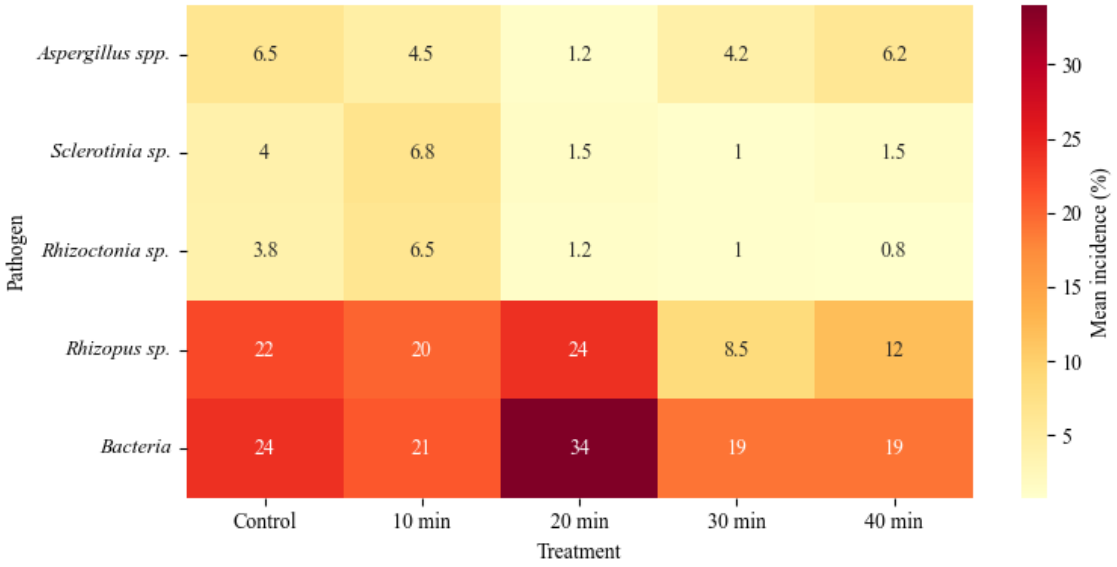


Figure 7. Heatmap of the mean incidence (%) of pathogens detected in soybean seeds exposed to UV-B radiation.

DISCUSSION

Ultraviolet-B (UV-B) produced contrasting physiological and sanitary responses that were governed by exposure time (cumulative dose). The stability of first germination count and seedling dry mass at ≤ 20 min indicates that moderate UV-B did not impair early vigor, which agrees with reports in soybean and other crops showing that calibrated doses can leave early establishment unaffected (Foroughbakhch Pournavab *et al*., 2019; Stefanello *et al*., 2023). By contrast, longer exposures depressed establishment, as reflected by the sharp declines in germination at 30 and 40 min, in line with time-dependent phytotoxic effects reported for diverse species (Foroughbakhch Pournavab *et al*., 2019; Ozel *et al*., 2021; Stefanello *et al*., 2023).

A prominent feature of the dataset was the peak in shoot and root length at 30 min. Although this pattern is compatible with a hormetic-like stimulation (Darras *et al*., 2019), it constitutes a clear case of survivor bias: elongation at 30 min occurred only among the seedlings that survived a treatment that simultaneously reduced germination to 44% (and to 28% at 40 min). These survivor-only gains do not translate into a net agronomic benefit and cannot offset the stand penalty at ≥ 30 min.

On the sanitary side, UV-B consistently increased the proportion of pathogen-free seeds and reduced total pathogen incidence. Here, "total incidence" denotes the percentage of seeds bearing at least one detectable pathogen in the blotter test; because multiple genera may co-occur on a single seed, the sum of per-genus incidences is not expected to equal this total (Goulart, 2018). Reductions were most evident for *Rhizopus* spp. and visible bacterial colonies. The likely mechanisms are dual: (i) direct effects on microbial structures (e.g., nucleic acids and spores), which reduce conidial viability under UV exposure (García-Cela *et al*., 2016; Ikram & Dawar, 2017), and (ii) host-mediated effects, whereby UV-B can modulate defense signaling and promote the accumulation of phenylpropanoid-derived compounds and phytoalexins, enhancing disease resistance when exposures are properly calibrated (Jenkins, 2009; Vanhaelewyn *et al*., 2016; Meyer *et al*., 2021; Loconsole & Santamaria, 2021). Together with prior observations of species- and dose-dependent responses in soybean and common bean (Stefanello *et al*., 2023; Hernandez-Aguilar *et al*., 2021; Al-Gabr *et al*., 2013; Foroughbakhch Pournavab *et al*., 2019), the present data support a selective sanitizing potential of UV-B against storage/opportunistic organisms.

Importantly, the blotter test enables detection of bacterial growth but not reliable taxonomic identification; our bacterial results are therefore reported as "visible colonies" only and should be interpreted as relative suppression rather than species-specific control (BRASIL, 2009; Goulart, 2018). Future work using selective media, biochemical assays, and/or molecular methods is needed to resolve bacterial and fungal taxa at species level and to refine epidemiological inferences (Meyer *et al*., 2021).

From an integrated disease-management perspective, 10–20 min emerges as a practical operational window: it preserves acceptable viability while initiating pathogen-load reduction. In commercial practice, such exposures could complement registered fungicide treatments potentially allowing lower chemical doses or serve as a standalone option in organic lots where synthetic inputs are restricted (Scariot *et al*., 2017; Nóbrega & do Nascimento, 2020; Rey *et al*., 2009; Loconsole & Santamaria, 2021). By contrast, 30–40 min should be regarded as high-intensity disinfection that achieves stronger sanitization at the cost of prohibitive stand losses and is therefore unsuitable for routine sowing. This positioning is consistent with broader non-chemical strategies that leverage calibrated UV regimes to enhance biotic resistance while avoiding vigor penalties (Mmbando, 2023; Winter & Rostas, 2008).

CONCLUSION

UV-B treatment of soybean seed entails a clear trade-off: sanitization improves as cumulative dose increases, whereas physiological viability drops sharply beyond 20 minutes of exposure. At the tested irradiance (1.5 W m^{-2}; 0.9–1.8 kJ m^{-2} for 10–20 min), the 10–20 minute interval provides the most practical balance initiating pathogen reduction while maintaining acceptable germination. Apparent elongation responses observed at 30 minutes occurred only among surviving seedlings and therefore do not translate into an agronomic benefit at the population level. Exposures of 30–40 minutes, although more effective for disinfection, are unsuitable for crop establishment due to severe losses in germination. Future work should (i) resolve fungi and bacteria to species level using culture-based and molecular methods, (ii) optimize dose delivery and irradiation geometry to ensure uniform seed surface exposure, and (iii) test UV-B in combination with biological control agents or with reduced-rate chemical fungicides within an integrated, non-chemical-forward disease-management program.

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AUTHOR CONTRIBUTIONS

Conceptualization: D.L.R.M. and N.P.C.E.; Data curation: N.P.C.E., D.L.R.M., E.J.B.G. and N.D.A.A.; Formal analysis: D.L.R.M., N.D.A.A. and N.P.C.E.; Investigation: N.P.C.E., D.L.R.M., E.J.B.G., L.S.R.V., N.D.A.A. and J.S.S.M.; Methodology: D.L.R.M., E.J.B.G. and N.D.A.A.; Project administration: D.L.R.M., L.S.R.V. and E.J.B.G.; Supervision: D.L.R.M. and J.S.S.M.; Validation: D.L.R.M. and J.S.S.M.; Writing – original draft: D.L.R.M. and N.D.A.A.; Writing – review & editing: D.L.R.M. and N.D.A.A.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this study.

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Supplementary ANOVA Tables

Table S1. ANOVA of Seed Germination Percentage of soybean seeds exposed to UV-B radiation.

Dependent variable: G (Germination)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	10484.20000	2621.05000	29.58	<.0001
Error	15	1329.00000	88.60000		
Corrected Total	19	11813.20000			

Table S2. ANOVA for Dead Seed Percentage of soybean seeds exposed to UV-B radiation.

Dependent variable: SM

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	350.8000000	87.7000000	5.93	0.0046
Error	15	221.7500000	14.7833333		
Corrected Total	19	572.5500000			

Table S3. ANOVA for Shoot Length of soybean seedlings derived from seeds exposed to UV-B radiation.

Dependent variable:: LPA

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	256.2770000	64.0692500	33.59	<.0001
Error	15	28.6150000	1.9076667		
Corrected Total	19	284.8920000			

Table S4. ANOVA for Root Length of soybean seedlings derived from seeds exposed to UV-B radiation.

Dependent variable: LR

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	362.7767500	90.694187593.32		<.0001
Error	15	14.728750	0.9819167		
Corrected Total	19	377.5055000			

Table S5. ANOVA for Seedling Fresh Mass of soybean seedlings derived from seeds exposed to UV-B radiation.

Dependent variable: MFPL

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	7.01538000	1.75384500	14.73	<.0001
Error	15	1.78650000	0.11910000		
Corrected Total	19	8.80188000			

Table S6. ANOVA for Pathogen-free Seeds (%) in soybean seeds exposed to UV-B radiation.

Dependent variable: SLP

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	5425.200000	1356.300000	41.60	<.0001
Error	15	489.000000	32.600000		
Corrected Total	19	5914.200000			

Table S7. ANOVA for *Sclerotinia* sp. colony counts in soybean seeds exposed to UV-B radiation

Dependent variable:: SCL

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	94.2000000	23.5500000	3.65	0.0287
Error	15	96.7500000	6.4500000		
Corrected Total	19	190.9500000			

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