

# Lentil Seed Germination and Stress Tolerance with UV-C Radiation: Physiological and Morphological Impacts Under Saline and Non-Saline Conditions

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**Abstract**—The aim of this study was to evaluate the effects of UV-C light on lentil (*Lens culinaris*) under two stress conditions: with and without salt (saline and non-saline). The main findings were: (i) Lentil showed significant changes ( $p < 0.05$ ) in the color dimension  $a^*$ , which decreased with increasing UV-C irradiation time. (ii) There were structural changes in the seeds due to UV-C degradation. With increasing irradiation time, more damage was observed in the seed cell wall. (iii) UV-C radiation produced positive and negative stimulatory effects depending on seed condition and irradiation time. The most significant changes ( $p < 0.05$ ) were observed 12 and 24 hours after the germination test. From 36 hours onward, the behavior of all UV-C treatments (0.0, 1.25, 2.5, and 5.0 minutes) and set stress conditions (saline and non-saline) and control samples started to show no variability. iv) Seedling weight increased by about 9% compared to control samples at 5 minutes of irradiation time. V) The correlation between fresh weight and color component  $a^*$  was inverse, i.e. the lower the value of  $a^*$ , the higher the seedling weight. UV-C could be a sustainable method for producing sprouts and improving the physiological quality of seeds once optimal UV-C irradiation times in and without salt stress conditions are defined.

**Index Terms**—UV-C, Germination, Degradation, Salinity, Physical methods

## I. INTRODUCTION

Salinity stress poses a significant challenge for global agriculture, exacerbating the urgency of innovative strategies to improve crop resilience in saline environments. Among the emerging nonchemical approaches, UV-C radiation has gained attention due to its potential to improve seed germination,

enhance plant stress tolerance, and induce biochemical and morphological adaptations critical for survival under saline conditions.

UV-C radiation has been extensively studied for its ability to modulate physiological and biochemical responses in plants. For example, Ouhibi et al. [1] and Alamer & Attia [2] demonstrated that UV-C treatment increases antioxidant activities and modifies phenolic compositions, thus improving salinity tolerance. Hernández-Aguilar et al. [3] found that UV-C treated lentils exhibited changes in phenolic content, linking UV-C exposure to potential biochemical modifications that could influence plant stress response mechanisms. Another study found that the integration of UV-C treatment with biostimulants, such as *Spirulina*, in the case of lentils shows the potential to mitigate damaging effects while boosting nutraceutical properties, thus improving both the resilience and nutritional profile of crops such as lentils, as in Ref. [4]. Similarly, studies on wheat and rapeseed have shown positive impacts on growth parameters and resilience to stress under saline conditions, as presented in Refs. [5]–[8].

Beyond its biochemical effects, UV-C has also been linked to seed morphological and structural changes. Hernández-Aguilar et al. [9] and Darras et al. [10] highlighted that low doses of UV-C irradiation stimulate growth and fruit yield by enhancing photoprotective metabolite production, which is crucial for plants under saline stress.

In addition, studies by Sadeghianfar et al. [11] emphasize that UV-C radiation can significantly improve seeds' germi-

nation rates and early growth metrics, such as radicle and plumule lengths, even in crops like maize and sugar beet, which are not typically associated with high salinity tolerance. This suggests that the beneficial impacts of UV-C radiation are broad and can be tailored to enhance crop resilience against different environmental stresses, including salinity.

The structural and physiological effects of UV-C radiation have also been documented. Joshi et al. [12] observed that lentils' cotyledon parenchyma cells are packed with protein bodies and starch granules, with the cell wall playing a critical role in holding reserves. Rupiasih and Vidyasagar [13] reported that exposure times longer than 180 minutes positively stimulated germination in wheat seeds, while Neelamegam and Sutha [14] found dose-dependent effects in groundnut seeds, with an initial increase followed by a decrease in germination at higher exposure times. These findings highlight the importance of optimizing UV-C exposure parameters for the desired results.

Previous studies have also explored the impact of UV-C radiation on specific crops. For example, Alamer and Attia [2] treated tomato seeds with UV-C and reported increased levels of chlorophyll, carotenoids, and anthocyanins, together with reduced salt stress aggressiveness in roots and leaves. Robles-Díaz et al. [15] demonstrated that UV-C irradiation improves water absorption in lentil and lupin seeds, probably due to structural changes in parenchyma cells and increased micropyle opening. These findings suggest that UV-C radiation improves germination and triggers secondary metabolite production, offering protection during early growth stages.

However, the effects of UV-C irradiation on germination are highly dependent on species, genotype, exposure time, and irradiance levels, as indicated in Ref. [16]. Kamel et al. [17] reported that UV-C exposure at 25 minutes stimulated germination and respiration rates in Apiaceae species, while Torres et al. [18] found that prolonged exposure reduced the percentage of normal seedlings in sunflower seeds.

Although positive responses have been documented in wheat [13] and Apiaceae species [17], other studies highlight inconsistent or even inhibitory effects. For example, groundnut (*Arachis hypogaea* L.) seeds showed an initial increase in germination followed by a decline depending on exposure time, as reported in [14]. Similarly, Hernandez et al. [3] found no significant statistical differences in bean germination after 15 minutes of UV-C exposure, although there was a tendency for germination to decrease. Furthermore, UV-C irradiation for 5 and 60 minutes reduced the percentage of normal seedlings in sunflower seeds, as analyzed in Ref. [18], demonstrating the importance of optimizing exposure conditions.

UV-C treatment has improved several physiological and biochemical traits in saline conditions. For instance, treating tomato (*Solanum lycopersicum*) seeds at doses of  $0.85 \text{ kJ.m}^{-2}$  and  $3.42 \text{ kJ.m}^{-2}$  significantly increased chlorophyll, carotenoids, and anthocyanin levels while mitigating salt stress damage to roots and leaves, as in Ref. [2]. This suggests that specific UV-C doses may activate the physiological stress response in plants, contributing to their resilience under saline

conditions.

In addition to germination enhancement, UV-C irradiation has been studied for its role in seed and sprout sanitation. UV-C has been considered a highly efficient and environmentally safe technology, Yang et al. [19], demonstrated that it can eliminate microbial contamination while preserving or improving seed's physiological quality. Such applications are particularly relevant in saline-affected environments, where UV-C may simultaneously strengthen plant defenses and reduce pathogen susceptibility, as in Refs. [20], [21].

Given the broad spectrum of UV-C's potential benefits, this study focuses on its application to lentil seed germination under saline conditions. By evaluating physiological parameters (e.g. dry weight, germination rate) and structural modifications, our research aims to provide deeper insights into how UV-C treatment could be optimized to improve seedling performance in saline-prone environments. Furthermore, this study considers UV-C treatment as a potential approach to enhance sprouts' agronomic and nutritional quality, aligning with the growing demand for sustainable and efficient agricultural practices in salt-affected regions.

## II. MATERIAL AND METHODS

### A. Biological Material

This study was carried out at ESIME-Zacatenco, Mexico City, in December 2024. The plant material used was lentils (*Lens culinaris Medik*) purchased in Mexico City (1®). The seed lot was standardized and the nutritional information of the lentils is as follows: protein = 9 g, dietary fiber = 8g, total fat = 0g, carbohydrates = 12g, and sugars = 2g.

### B. Treatment of UV-C Radiation

The lentils were treated prior to planting using a UV-C sterilization system (STERI BAG 3W, Bright Solutions) depicted in Fig. 1. This system incorporates a single UV-C lamp mounted on the interior of the unit lid, emitting a wavelength of  $253.7 \text{ nm}$  with a power output of 3.50 W. The sterilization unit is compact, measuring 12 cm in height, 14 cm in width, and 24 cm in length, which makes it suitable for small-scale applications. The device operates at a 5V DC input voltage with a rated power of 3.50 W and has a maximum capacity of 3.8 L. This setup was used to expose the lentil seeds to UV-C radiation for predetermined intervals of 0.0, 1.25, 2.5, and 5.0 minutes, similar to the protocols used in the study mentioned by [10]. The light intensity measured with UV-C / 254 measurement equipment was  $400 \text{ mW/cm}^2$ .

### C. Germination Test

A germination test was conducted using a complete randomized block design with five replications. Each replicate consisted of 20 seeds, defined as an experimental unit. Following UV-C treatment, the seeds were sown in Petri dishes. Each dish was lined with moistened filter paper. Two hydration treatments were applied: Lot B was moistened with 5 ml of distilled water; Lot A used a saline solution, consisting of a 0.75% sodium chloride (NaCl) concentration, equating to 7.5g



Fig. 1: UV-C system used to irradiate lentil seed in December 2024.

of NaCl (SAL SOL Sea salt) per 1000ml of distilled water. This setup was intended to evaluate the effects of saline stress and the impact of UV-C radiation on seed germination.

The germination stage was considered finished 5 days after the experiment was established. The germinated seeds were counted every 12h until germination (G0, G12, G24, G36, G48, G60, G72, G84, G96, G108) became uniform (five days). The temperature and humidity conditions corresponded to an average temperature of 14C and 60% humidity. At the end of the germination, fresh weight of seedlings was obtained.

#### D. Color

The color of the different lentil treatments (0, 1.25 and 2.5 min) was determined with a portable colorimeter (FRU WR-10QC, Shenzhen Wave Optoelectronics Technology Ltd, China). The color parameters measured were those corresponding to the CIELAB uniform color space ( $L^*$ ,  $a^*$  and  $b^*$ ) were obtained directly from the instrument.  $L^*$  indicates lightness (100 = White and 0 = Black), “a” indicates greenish-reddish [negative ( $-a$ ) (green) to red ( $+a$ ) (positive)] and “b” indicates blue-yellowish [negative ( $-b$ ) (blue) to yellow ( $+b$ ) (positive)]. The equipment was calibrated using a reference blank and white. Color measurements were taken on lentil seed samples before and after the radiation process.

#### E. Methodology of high-vacuum scanning electron microscopy

The morphological changes of the lentil seeds in different UV-C treatments (0.0, 1.25, 2.5 and 5.0min) were analyzed in a high vacuum scanning electron microscope (JEOL JSM-6010LA, Tokyo, Japan). Before analysis, the lentil seeds were cut in half and placed in an aluminum sample holder and fixed with carbon tape. The samples were coated with gold nanoparticles. Micrographs were taken at 1000x, analyzing the parenchyma zone where the starch, the cell wall, and the protein body are located. The analysis conditions on the equipment were 15 kV electron accelerating voltage.

#### F. Statistical analysis and principal component analysis of the lentil color

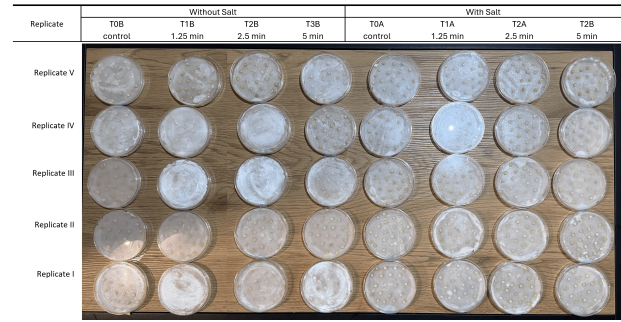
The variables lentil color before and after irradiation at the exposure times used, the germination of seeds evaluated every 12hours and the fresh weight measured at the end of the test were statistically analyzed by analysis of variance ( $p < 0.05$ ) (ANOVA) and the least significant difference (LSD) test at the probability level 5% was used to compare treatments [22].

The software used for the analysis was SAS [23]. PCA was applied to the experimental data obtained from the germination variables (G0, G12, G24, G36, G48, G60, G72, G84, G96, G108) and seedling weight evaluated at the end of the test.

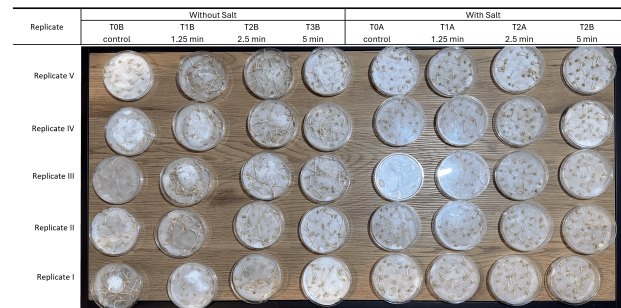
### III. RESULTS

#### A. Germination

Fig. 2 shows the germination stage at the beginning (G0) of the experiment (Fig. 2a) and at the end of the experiment (G108, Fig. 2b).



(a) Germination start stage: G0



(b) Germination end stage: G108

Fig. 2: Germinated seeds: a) Stage at 0 hours. b) Stage at 108 hours.

According to the analysis of variance and the comparison of means between the treatments established in the germination test. Significant statistical differences were found for the variables of germination seedling weight, G12 and G24 ( $p < 0.05$ ).

Fig. 3 shows that the saline and nonsaline conditions have a significant increase in dry weight at the irradiation time of 5.0min. It is also observed that the control samples for both conditions (A and B) show similar behavior, indicating the resistance of lentils to salinity stress conditions. In the saline condition, no changes are observed at the times of 1.25 and 2.5min, that is, despite the irradiation, its behavior remains stable in the weight variable. In the case of the lentil without salt (B), at the first irradiation times of 1.25 and 2.5min, it is observed that it tends to decrease, that is, stress is perceived in the seed. However, after 5 minutes, the dry weight increases. In both saline and non-saline conditions, the increase is about 9% with respect to the control samples.

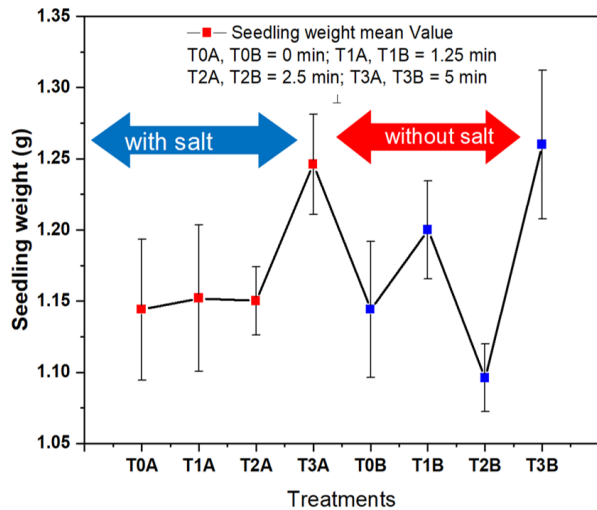


Fig. 3: Effects of UV-C radiation on the fresh weight of lentil seedlings that were pre-sowing treated.

Figs. 4a and 4b show the kinetics of germination percentage of lentil seeds with UV-C light treatment (0.0, 1.25, 2.5 and 5.0min) pre-sowing obtained every 12hours. Figure 4a shows the results of the measurement 12hours after the experiment was established until 108 hours after it was established. The highest differences in germination occur in the first 24hours. The germination percentage of the lentil seeds begins to homogenize from the measurement taken at 36h. Figure 4b shows the behavior of germination percentage of lentil seeds in the early stages of germination (12, 24 and 36h). It is observed that in the saline condition, germination increased at 1.25 and 2.5 min. However, in the non-saline condition, germination decreased for the times of (1.25, 2.5 and 5min).

Fig. 4b shows how the germination percentages of the samples in condition (A) at 12 and 24 hours are below those of the samples in condition without salinity (B). In general, at 12 and 24 hours, condition B presents a greater range of variation in the germination percentage in relation to samples under saline condition. It is worth mentioning that in the saline condition, the control samples and those treated with UV-C at 1.25 and 2.5 minutes have a similar behavior. However, at a radiation time of 5 minutes, the germination percentage tends to decrease.

#### B. Statistical analysis and PCA

The color data were analysed using ANOVA and LSD test at the 5% significance level. Table I shows that there were no statistically significant differences in the  $L^*$  and  $b^*$  color components. However, the  $a^*$  component decreased significantly ( $p < 0.05$ ) with increasing radiation time, particularly after 2.5 minutes of exposure.

Fig. 5 shows the results of the PCA, indicating the evaluated variables, where it is observed that the smaller the angle formed between the vectors representing the variables, the higher the correlation between them. It is important to note

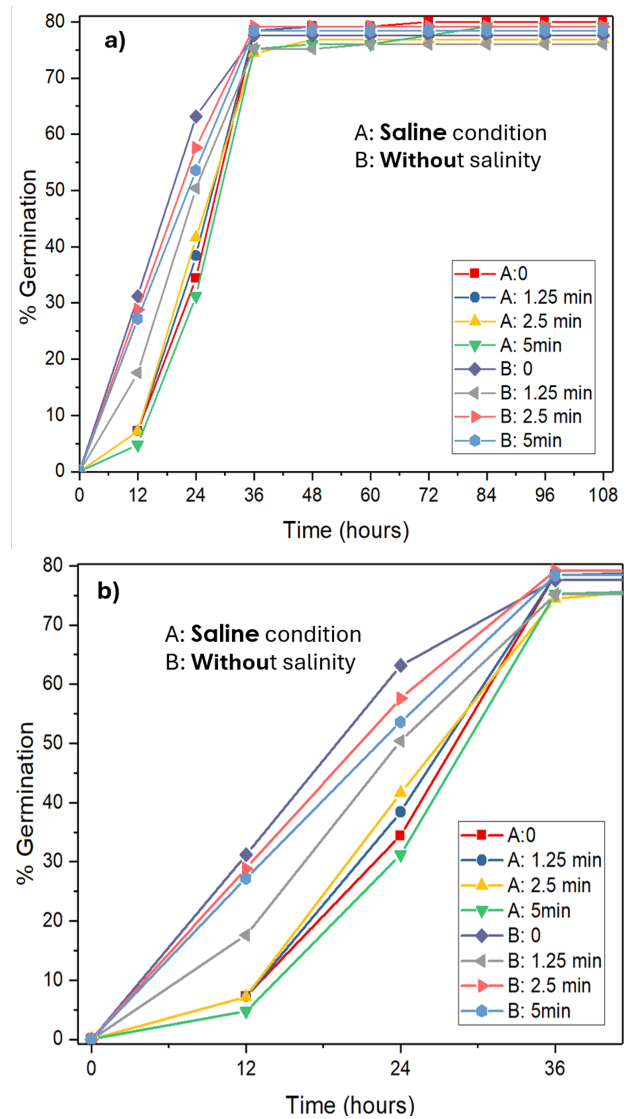


Fig. 4: Kinetics of germination percentage of lentil seeds conditioned for germination with UV-C light (0, 1.25, 2.5 and 5 min). a) Germination percentage every 12 hours b) % germination at 0, 12 and 24 h.

that the fresh weight of the seedlings is inversely correlated with the color component  $a^*$ . The lower the value of  $a^*$ , the higher the fresh weight. There is more correlation between germination between 36 and 108 hours, as confirmed in the graphs described above. This is when it tends to stabilize the emergence of lentil seed seedlings, reaching its maximum value of germinated seeds at 72 hours.

#### C. Morphological changes in UV-C irradiated seeds

Fig. 6 shows that the cells of the cotyledon parenchyma are packed with protein bodies and starch granules. The structure that holds the reserves is the cell wall, which is composed of cellulose, hemicellulose, pectin, and lignin. Joshi et al. [12] also observed a similar structure in lentils using microscopy. The images show that the starch granules in the seed are



TABLE I: Comparison of color variables ( $L^*$ ,  $a^*$  y  $b^*$ ) of lentil seeds with different UVC treatments

Exposure time	$L^*$	$a^*$	$b^*$
0	51.59a	6.09a	23.32a
1.25	51.67a	4.65ba	22.05a
2.5	53.55a	3.87b	20.06a
5	53.29a	3.46b	22.93a
DMS	2.02	3.46	2.74
C.V	3.15	29	10
Significance	0.11ns	0.01*	0.08bs
$R^2$	0.51	0.54	0.47

Means with different letters in a column by type of treatment are statistically different ( $p < 0.05$ ). ns: there were no significant differences, (DMS,  $\alpha = 0.05$ ). \* Significant at 5 and 1% probability

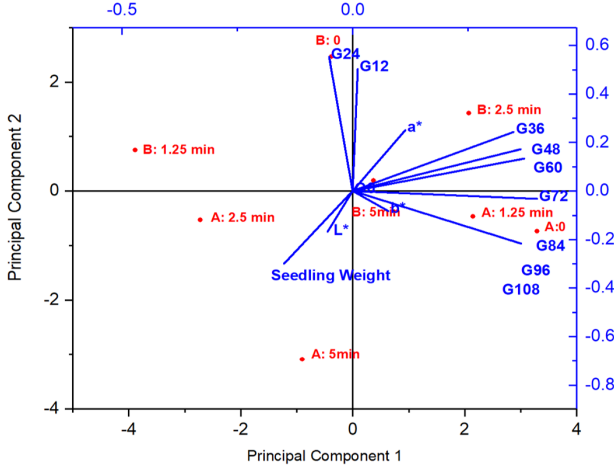


Fig. 5: Principal component analysis of germination variables evaluated a) saline condition and b) no-salinity condition.

mostly spherical. When UV-C was applied to the seeds for 1.25 min, no changes were observed in seed T1. However, as the irradiation time increased, more damage was observed in the cell wall of the seeds, even at T3, there were ruptures, and the starch granules were detached from the packed body. This figure also shows that some cell wall lamellae begin to show what could be cellulose and hemicellulose microfibrils.

#### IV. DISCUSSION

In the present study, seed treatment with UV-C light was found to modify physiological quality variables, color, and morphology, both in saline and non-saline conditions established during seed germination. The specific timepoints evaluated in the study were: 36, 48, 60, 72, 84, 96, and 108 hours. Although the main applications of UV-C light have been reported in the sterilization of food, surfaces, and air, there are applications in the agricultural sector, for example, in the different attributes of seed quality (sanitary, physiological, and nutritional) that could have great potential. The effects of UV-C light have been observed at the seed and germination levels.

The present research found that UV-C light induces seed degradation at the seed level, causing structural changes as a function of exposure time. Thus, the irradiation time parameter

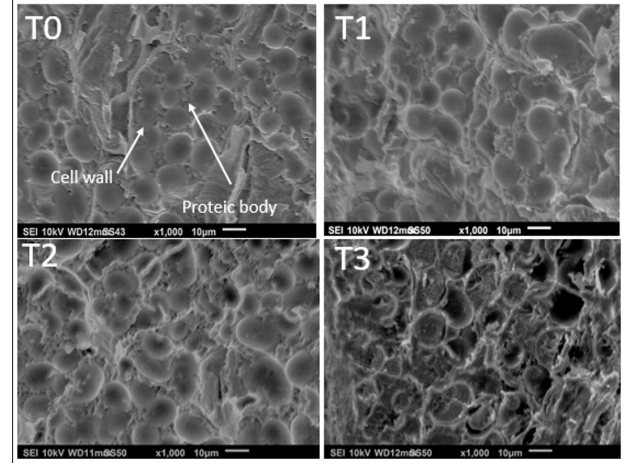


Fig. 6: Micrographs of lentils treated with UV-C radiation ( $T_0 = 0min$ ,  $T_1 = 1.25min$ ,  $T_2 = 2.5min$ , and  $T_3 = 5.0min$ ).

is relevant to processes for improving quality attributes and their development under conditions of saline or hydric stress due to the increase of bioactive elements in plant protection.

At the level of the germination effect, it can be said that it was affected in its initial stages by the saline condition, being reduced from 30 to 5 and 60 to 30%, respectively, at 12 and 24 hours after sowing (control samples). It is important to note that this variable of germination percentage (considering a germinated seed when it has a minimum radicle length of 0.5 cm) becomes almost uniform after 36 hours, since the variation found was approximately 5% between the samples under saline or non-saline conditions and irradiated and non-irradiated with UV-C light at the evaluated times of 36, 48, ... 108 hours.

It should be noted that the difference in germination percentage between the samples in the saline condition (A) and the treatment that caused the highest negative stimulation ( $t = 5.0min$ ), and the treatment of the control sample without salt stress and the treatment that caused the highest negative stimulation (1.5 and 5.0min) was lower in the samples under the saline condition (2%) than in the condition without salt stress (approx. 10%).

UV-C radiation has been reported as a strategy to increase seed germination under salt and non-salt stress conditions at different UV-C radiation times and intensities. Several authors have reported that the effects of UV-C radiation can be positive, negative, or null depending on the radiation parameters, e.g., time, and whether the radiation is applied short or long.

Rupiasih and Vidyasagar [13] reported the application of exposure times longer than 180 min to wheat seeds (variety: Lok-1), which resulted in positive stimulation of germination. Positive stimulation has also been found by other authors, but negative stimulation has also been found.

In groundnut seed (*Arachis hypogaea* L.) with UV-C, the authors found an increase and then a decrease depending on the time applied 5 or 60 min [14]. In previous studies,

UV-C was applied to bean (*Phaseolus vulgaris*) seeds for 15 min at different irradiance, using a prototype integrating  $4 \times 60$  W lamps and an intensity of  $700 \text{ W cm}^{-2}$ . There were no significant statistical differences between the treated and control samples. However, germination decreased [3]. Other growth parameters, such as the percentage of normal seedlings, were also evaluated.

The exposure of UV-C to sunflower seeds at 5 and 60 min was reported to reduce the percentage of normal seedlings [18]. Other negative and positive effects have been reported depending on the seed species, its genotype and radiation source [16]. The *aphiaceae* species were treated with UV-C at an intensity of  $10.5 \text{ mW/cm}^2$  and an exposure time of 0-45 min, with 5 min increments; In a germination test, it was reported a time of 25 min stimulated seed germination and respiration rate compared to control samples [17].

In the present study, it was found that the physiological quality variable improved was dry weight. In this case, the seed dry weight increased by about 9% at 5 min, both for the saline and non-saline conditions.

In saline conditions, several variables have been improved by UV-C radiation under salinity stress conditions. Alamer and Attia [2] treated tomato (*Solanum lycopersicum*) seeds with 60 W UV-C lamps at doses of  $D1 = 0.85 \text{ kJ.m}^{-2}$  and  $D2 = 3.42 \text{ kJ.m}^{-2}$  and reported increased levels of chlorophyll, carotenoids, and anthocyanins. However, they also reported that UV-C light appeared to control the aggressiveness of salt stress on tomato roots and leaves. They pointed out that specific doses of UV-C are required to activate the physiological control of plants against salt stress.

In the present study, it appears that UV-C reduces the effects of salt stress in treated plants, in the variable fresh weight of seedlings, since it has a value in the same order as the lentil that was not stressed under saline conditions. Thus, there are mechanisms of action of UV-C light that modify the seed and seedling variables in germination.

Robles-Díaz et al. [15] showed that water absorption in lentil and lupin seeds occurs preferentially through the micropyle, which is assumed to increase with the times of UV-C irradiation, since the opening of the micropyle is greater, causing greater structural changes in the seeds parenchyma cells. In the present study, the level of irradiance applied and the time of irradiation modified the structure of the seed, probably allowing greater water uptake. In addition, salt stress and UV-C stress are likely to trigger the production of secondary metabolites that protect the seed and its early germination stages. Thus, along with other physical methods such as magnetic field, laser technology, LED light and others, are presented as alternatives to improve seed quality in its various attributes.

It is essential to mention that there are several benefits that can be obtained from the treatment of seeds and sprouts, the UV-C irradiation method could be used to improve seed germination, as an increase in fresh weight was found. In addition, according to the literature, the microbial quality of the seed could be guaranteed since it is a sustainable technology. As

indicated by Yang et al. [19], UVC radiation, among other physical methods, has high efficiency and environmental safety characteristics.

## V. CONCLUSIONS

UV-C radiation as seed pretreatment modifies the physiological quality components in germination and fresh weight variables, in both saline and non-saline conditions. Significant changes in germination were found in the early stages of germination at 12 and 24 hours. Concerning the weight of the seedlings obtained, an increase of 9% was found in the saline (A) and non-saline (B) conditions when the radiation treatment was applied 5 minutes.

UV-C radiation produces morphological changes in the lentil. The radiation effect damages the cell wall and the protein body, which begins to manifest itself after 5 minutes. No damage to the starch is visible.

UV-C radiation degrades and modifies the seeds, mainly affecting the  $a^*$  color component. For irradiation times of 2.5 and 5 minutes, the color component decreased significantly between 36 and 43%.

Although this research confirms the beneficial effects of UV-C radiation on seed quality, further research is required to establish its long-term impact on plant development, biochemical pathways, and crop yield. Future studies should explore optimal exposure parameters across different plant species, analyze gene expression changes associated with stress tolerance, and assess field-scale implementation in saline and non-saline environments. In addition, integrating UV-C treatment with complementary agricultural technologies, such as microbial inoculants or biostimulants, could enhance its applicability for sustainable agriculture.

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