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Environmental Technology

Does ultraviolet radiation affect the germination of Persian clover seeds?

A radiação ultravioleta afeta a germinação de sementes de trevo Persa?

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ABSTRACT

This study aimed to evaluate the influence of ultraviolet light on the germination of *Trifolium resupinatum* L. seeds (Persian clover). The seeds were exposed to different times (doses) of exposure to UV-B and UV-C light with radiation intensity of 2.5 W m^{-2}. Afterward, they were sown in gerbox boxes with germitest paper and stored in a Biochemical Oxygen Demand at 20°C. Tests such as germination, germination speed index, length and dry mass of seedling were performed. No significant differences were among UV-B light treatments. However, seeds exposed to 8 hours of UV-C light showed a reduction in the germination speed index when compared to control (from 92.5 to 88.7). Furthermore, the length of the seedlings increased by an average of 1.3 cm in relation to the control. Under the conditions of this study, it was concluded that Persian clover seeds exhibit relative tolerance to UV-B radiation while showing some sensitivity to UV-C radiation. The pelleting of the seeds associated with the tegument acted as a physical block, impeding the passage of UV light. The results contribute to our understanding of how Persian clover responds to UV radiation exposure.

Keywords: Light; Process of germination; *Trifolium resupinatum.*

RESUMO

O objetivo deste estudo foi avaliar a influência da luz ultravioleta na germinação de sementes de *Trifolium resupinatum* L (trevo Persa). As sementes foram submetidas a diferentes tempos (doses) de exposição à luz UV-B e UV-C e intensidade de radiação 2.5 W m^{-2}. Em seguida, foram semeadas em caixas gerbox com papel germitest e armazenadas em câmara Biochemical Oxygen Demand, a 20ºC. Foram realizados os testes de germinação, índice de velocidade de germinação, comprimento e massa seca de plântulas. Não foram observadas diferenças significativas entre os tratamentos com luz UV-B. No entanto, as sementes expostas a 8 horas à luz UV-C apresentaram redução no índice de velocidade de germinação quando comparadas ao controle (de 92,5 para 88,7). Além disso, o comprimento das plântulas apresentou aumento médio de 1,3 cm em relação ao controle. Nas condições deste estudo, concluiu-se que as sementes de trevo Persa apresentam relativa tolerância à radiação UV-B, embora apresentem alguma sensibilidade à radiação UV-C. A peletização das sementes associada ao tegumento atuou como um bloqueio físico, impedindo a passagem da luz ultravioleta. Os resultados contribuem para a compreensão de como o trevo Persa responde à exposição à radiação UV.

Palavras-chave: Luz; Processo germinativo; *Trifolium resupinatu*

1 INTRODUCTION

Currently, the growth of the world population is considered the cause of many significant problems worldwide. Consequently, it has caused excessive use of the planet's limited resources. This process has exerted a significant amount of pressure on the ecosystem. It has generated worrying problems such as environmental pollution, increased concentrations of CO_2 in the atmosphere, global climate change, a decrease in the living spaces of many living beings, and, consequently, the loss of species (Ozel et al., 2021).

One of the global impacts of this process is the depletion of the stratospheric ozone layer (at a height of 10 to 30 km around the world), which allows solar radiation to penetrate the atmosphere and reach the Earth's surface more quickly. As a result of the indiscriminate use of environmental pollutants such as chlorofluorocarbon gas, more ultraviolet radiation reaches the Earth's surface (Inostroza-Blancheteau et al., 2016). Rising ultraviolet (UV) radiation levels can harm all life forms, including plants, animals, and even microorganisms (Shaukat et al., 2013). Studies indicate that these rays can cause harmful effects on plants, including changes in genomics, oxidative damage, lipid destruction, biochemical changes, and reduced growth (Neugart and Schreiner, 2018). These UV-induced effects generally depend on the intensity of radiation and the stages of plant development (Moreira-Rodriguez et al., 2017).

In this context, important research has been conducted on the subject. These studies suggest that UV-B and UV-C light can have a positive or negative influence on seed germination and seedling development of several species (Shaukat et al., 2013; Neelamegam and Sutha, 2015; Pournavab et al., 2019; Sadeghianfar et al., 2019; Tripathi et al., 2019; Lazim and Ramadhan, 2020; Vanhaelewyn et al., 2020; Hernandez-Aguilar et al., 2021; Ozel et al., 2021; Semenov et al., 2021).

However, despite extensive experimental data, no basic or applied research was found on the physiological responses of plants to UV radiation for the species *Trifolium resupinatum* L. (Persian clover). This species belongs to the Fabaceae family and is an annual legume, growing prostrate or semi-upright with a branched structure, reaching

20–60 cm in height (Heuzé et al., 2015). It is mainly utilized for forage, providing highly nutritious and palatable pasture and hay. Depending on the variety, its high moisture and protein content might make it unsuitable for silage. It stands out because it can grow in areas with high soil moisture and poor drainage. It constitutes an excellent alternative for grazing, with high regrowth capacity after cutting (Sganzerla et al., 2015).

In this context, considering the hypothesis that high concentrations of ultraviolet light can affect seed germination, the goal of this study was to evaluate the impact of UV-B and UV-C radiation on the germination of Persian clover seeds, which are considered a model crop due to their current rapid germination and development.

2 MATERIALS AND METHODS

The pelleted seeds of *Trifolium resupinatum* L., cultivar Lightning, crop 2021, were acquired from a traditional company involved in the commercialization of seeds (PGG Wrightson Seeds Brasil).

Before every procedure, the lamps were on for five minutes. Then, the seeds were placed in Petri dishes and inserted into an irradiation chamber with a lamp emitting UV-B radiation (Ushio G15T8E) at a distance of 25.5 cm from the lamp for different intervals of exposure to the light (0, 1h, 2h, 4h, and 8h), according to Table 1. At a distance of 34 cm, the identical operation was carried out using a UV-C radiation lamp (Philips TUV 15WG15T8). The UV-B radiation intensity was calibrated using a UV Monitor MS-211-1 from EKO Instruments Co., Ltd., and the UV-C intensity was calibrated with a UV-C 254 radiometers from Lutron. It was essential to adapt the distance between the base and the UV-B and UV-C lamps to ensure that the seeds got the same amount of radiation intensity (2.5 W m^{-2}).

Table 1 – UV-B and UV-C doses and exposure times for Persian clover seed germination in a constant irradiance chamber

Exposure time (hour)	Dose (J m^{-2})	Dose (mJ cm^{-2})	Dose (KJ m^{-2})
0	0	0	0
1	9000	900	9
2	18000	1800	18
4	36000	3600	36
8	72000	7200	72

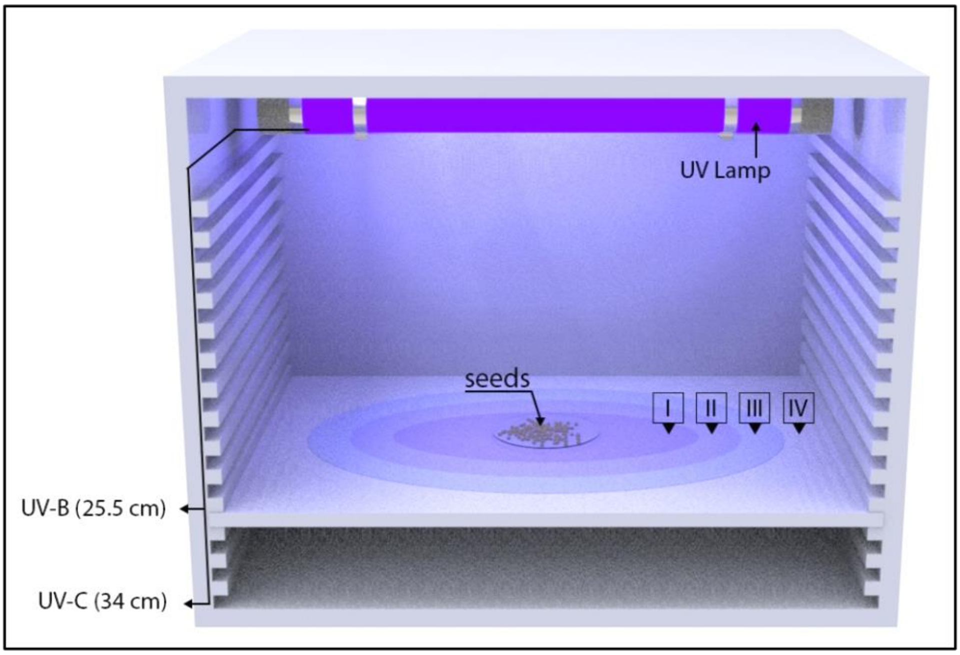
Source: Authors (2023)

The seeds were placed inside a glass Petri dish as evenly as possible to avoid overlapping each other and ensure that they were reached by the same intensity of radiation. The intensity of UV radiation was measured in different regions of the irradiation chamber using radiometers (models described above). The constant and highest intensity values were observed in the central region. The Petri dish with the seeds was placed in the central part of the chamber during the exposure times (treatments), ensuring that they were reached by the same intensity of UV light (Figure 1).

The following studies were conducted to assess the physiological potential of the seeds subjected to varying amounts of UV light:

Germination test (%): conducted based on four repetitions of 50 seeds distributed in plastic boxes (gerbox) on germitest paper moistened with distilled water (2.5 times the weight of the paper). The intensity of UV radiation was measured in different regions of the chamber, with the constant and highest intensity observed in the central region. Therefore, it was necessary to reduce the number of seeds (using the adapted methodology of Brasil, 2009) so that all could receive the same intensity in the reduced space. After sowing the seeds, the plastic boxes were maintained in BOD chambers (Biochemical Oxygen Demand) at a constant temperature of 20°C and 12 hours of light. Counts were made on days seven (Brasil, 2009).

Figure 1 – A representative scheme irradiation chamber shows seeds at the bottom and a UV lamp at the top. The indicators UV-B (25.5 cm) and UV-C (34 cm) on the left side show the level position where the seeds are left before installing the UV lamp (B or C). The intensity gradually decreases away from the center in the four areas (I, II, III, and IV) on the bottom center (I = 2.5 W m^{-2}, II = 2.1 W m^{-2}, III = 1.8 W m^{-2}, and IV = 0.7 W m^{-2})



Source: Authors (2023)

Germination speed index (GSI): evaluated with germination, counting the emerged seedlings daily (Krzyzanowski et al., 2020).

Seedling length (cm): four replications of 20 seeds were sown in two unmatched rows in the upper third of the germination paper and maintained under the same conditions as the germination test. On the fourth day after sowing, the lengths (shoots and roots) of ten normal seedlings from each replication were measured.

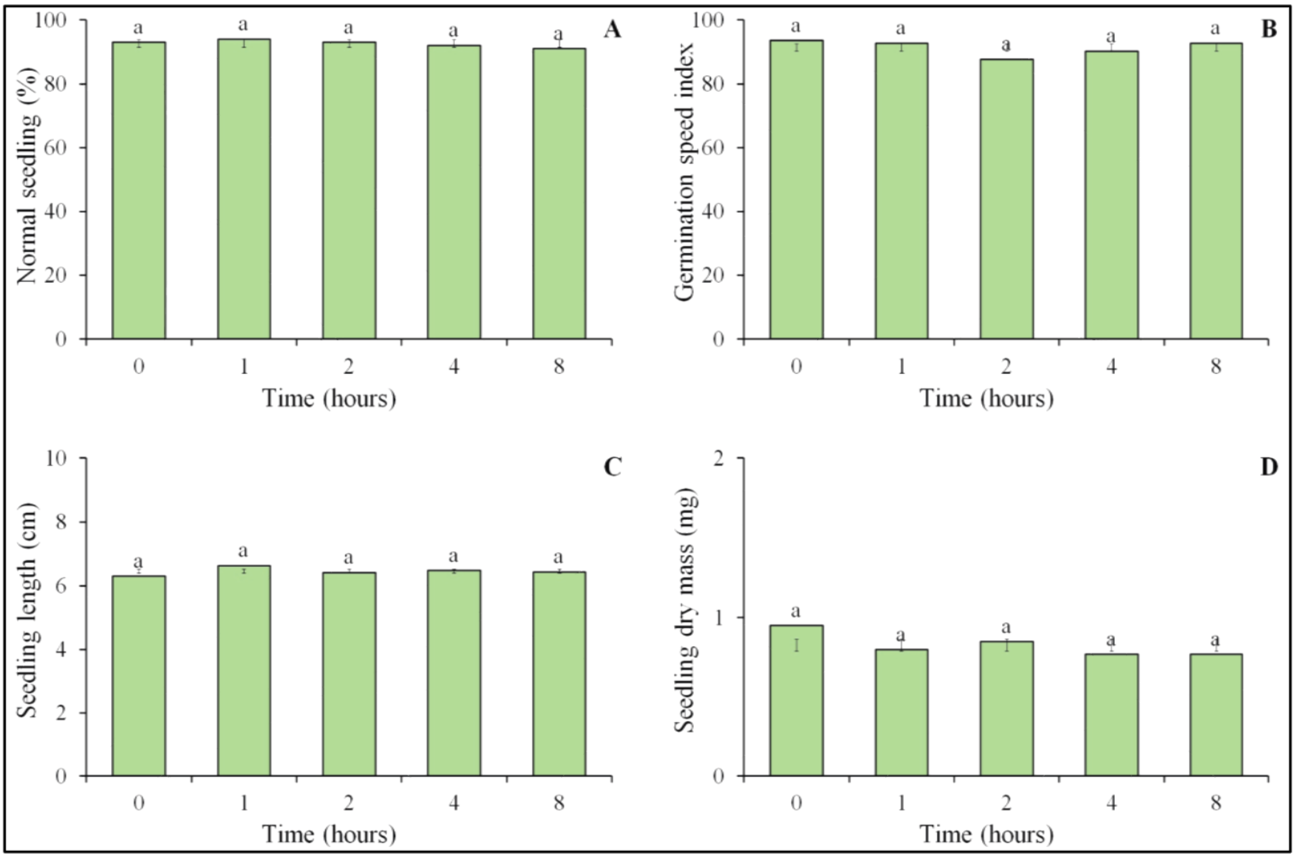
Seedling dry mass (mg): ten normal seedlings from each replication of the seedling length test were selected. The seedlings were weighed on a precision balance (of 0.001 g) after drying the material in a forced ventilation oven at 60 ± 5°C for 48 h.

The experimental design was totally randomized. The two treatments consisted of exposing the seeds to light (UV-B and UV-C separately) at five different times (doses) and four replications per treatment. The data were submitted to an analysis of variance using the F ($p \leq 0.05$) test, and, when significant, a regression analysis was performed using the program Sisvar software version 5.6. The presentation of column graphs was chosen for better viewing of the results.

3 RESULTS AND DISCUSSION

Through the analysis of the data, no significant difference ($p > 0.05$) was observed in the variables such as germination, first count, germination speed index, length, and dry mass of Persian clover seedlings (Figure 2). The average germination rate ranges from 93% (control) to 91% (8 h) (Figure 2A).

Figure 2 – Germination (A), germination speed index (B), total length (C), and dry mass (D) of Persian clover seedlings exposed to UV-B radiation

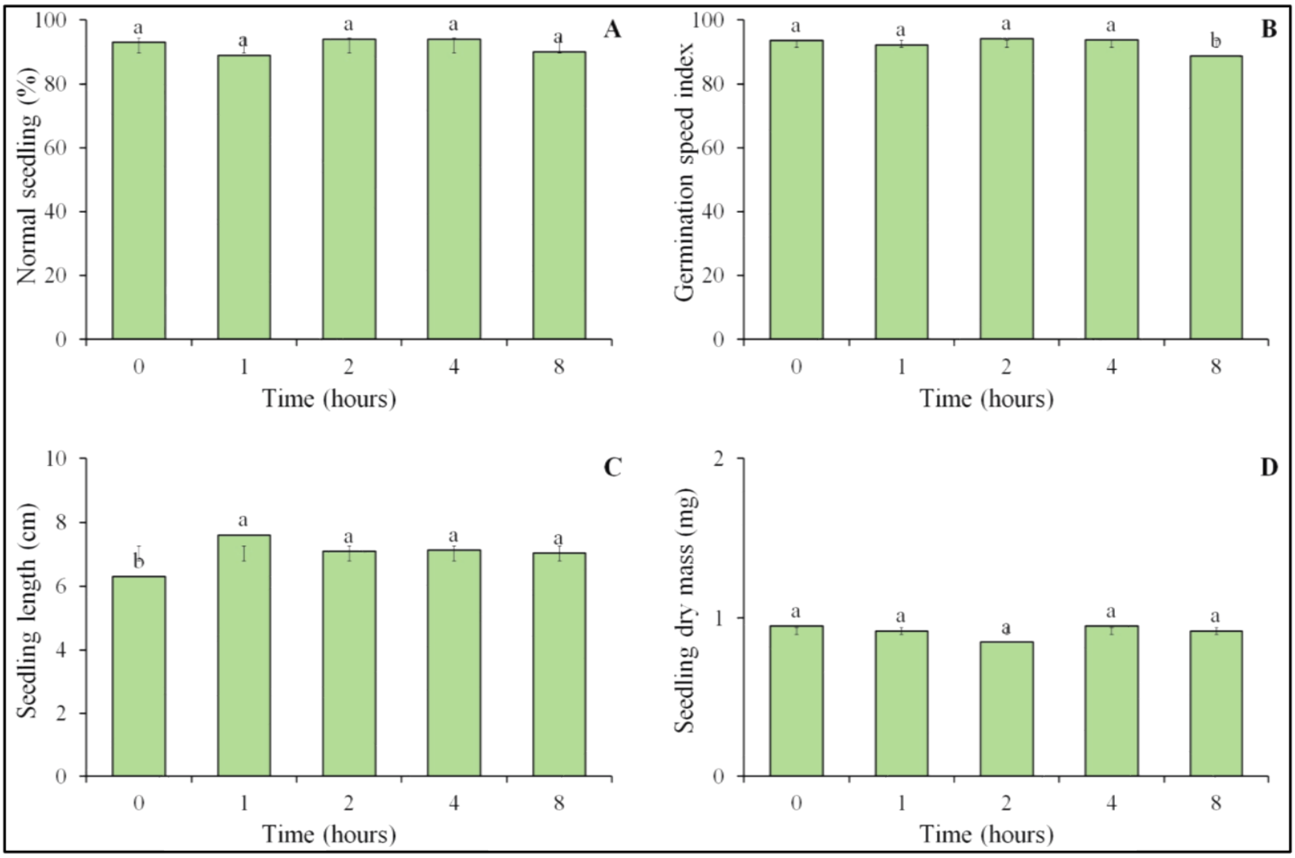


Source: Authors (2023)

When the data about UV-C exposure was analyzed, it was verified that there was a significant difference ($p < 0.05$) in the germination speed index and total length of seedlings as a function of the treatments (time of exposure to light), as shown in

Figures 3C and 3D. On the other hand, there was no difference in variables such as germination and dry mass of Persian clover seedlings (Figures 3A and 3D).

Figure 3 – Germination (A), germination speed index (B), total length (C), and dry mass (D) of Persian clover seedlings exposed to UV-C radiation



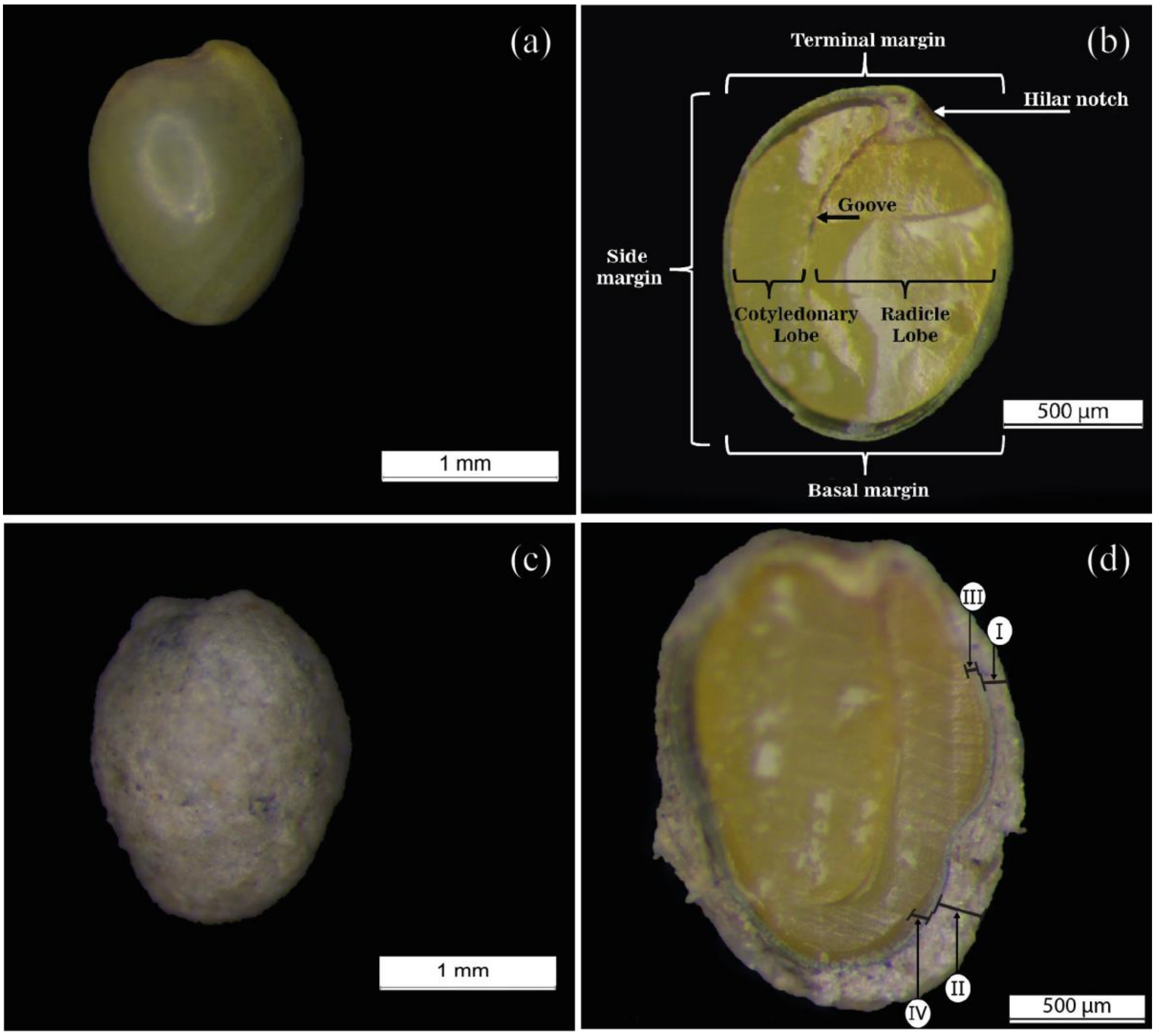
Source: Authors (2023)

Seeds exposed to UV-C light for 8 hours reduced germination speed compared to the control (from 92.5 to 88.7) (Figure 3B). In all treatments, the length of the seedlings increased by an average of 1.3 cm compared to the control group (without exposure to light) (Figure 3C). UV-C radiation exhibited a minimal effect on Persian clover germination and initial growth at exposure times of up to 8 hours (doses up to 72000 J m^{-2}).

Previous studies on this subject indicate that different plant species exhibit distinct responses to varying stress levels, which may be related to the plant's morphological characteristics (Aricak et al., 2019; Sevik et al., 2020). Plant surface characteristics have a significant impact on UV-C radiation effectiveness, as the presence of cracks, cavities, and other irregularities, in addition to surface roughness, can attenuate incident energy (Balbinot Filho and Borges, 2020).

According to the results of this study, it is possible to infer that the seed pelleting (adherent solution + specific *Rhizobium*) associated with the integument composed of palisade cells with thick walls and externally covered by a waxy cuticular layer acted as a physical block, making the integument resistant (Agra et al., 2016) and, consequently, hindering the passage of UV light (Figure 4).

Figure 4 – Overview of Persian clover seeds: non-pelleted (a) and cross-section with internal structures (b); pelleted seed (c) and cross-section with seed and pellet thicknesses (I: 0.105 mm, II: 0.214 mm, III: 0.023 mm, and IV: 0.045 mm) (d)



Source: Authors (2023)

Although high-dose UV light is known to spoil quality and production parameters, some studies indicate that low-dose ultraviolet light can stimulate biomass accumulation and the synthesis of compounds that mainly absorb UV light (Loconsole and Santamaria, 2021). According to Meyer et al. (2021), UV-B light operates in two ways, depending on the dose. Low doses of UV-B light can help the plant's defense systems, while high doses can be harmful because they can inhibit growth and change the morphological characteristics of plants. When exposed to UV radiation, plants may initiate several physiological and morphological changes that result in substantial differences between species and even within the same species (Neelamegam and Sutha, 2015).

In agriculture, continuous non-ionized UV-C radiation has been applied to different fruit, vegetable, and leguminous crops and has been shown to have a positive impact on the germination and vigor of fruit crops (Sukthavornthum et al., 2018). Employing ultraviolet-C radiation on seeds such as peanuts (Neelamegam and Sutha, 2015), and vetch (*Vicia villosa* Roth) (Semenov et al., 2021), increased germination and growth parameters. Likewise, Changjan et al. (2022) analyzed *Ipomoea aquatica* Forssk seeds exposed to UV-C (254 nm) for periods of 2 (1.4 $J.cm^{-2}$), 4 (2.9 $J.cm^{-2}$), 8 (5.8 $J.cm^{-2}$), and 12 (8.7 $J.cm^{-2}$) hours and observed an increase in germination rate, root length, and root thickness after 2 hours of irradiation.

Exposure to UV-B and UV-C has harmful effects, often resulting in the death of the microorganism. Therefore, it is interesting for an agricultural application so long as the plant or beneficial organisms are less UV-damaged than their natural pathogen (Vanhaelewyn et al., 2020). To be effective, the UV radiation must reach the microorganism directly to eliminate it. High turbidity in liquids can reduce the effectiveness of UV-C sterilization (Jones et al., 2014). UV-C is more effective than UV-A and UV-B at reducing the infection pressure of plant pathogens. This is managed by a method comparable to surface sterilization. Short treatments with UV-C eliminate microorganisms while the plant remains unharmed.

The preliminary data from this study is promising. However, more research is needed to fully understand the mechanisms underlying plant responses to UV radiation and other abiotic factors, as well as the various factors that interact and induce plant responses to changes in environmental conditions. Besides, investigating UV-induced anatomical damage is significant and may provide new information about the species and new data for the literature.

4 CONCLUSIONS

Under the conditions of this study, it was concluded that Persian clover seeds exhibit relative tolerance to UV-B radiation while showing some sensitivity to UV-C radiation. The pelleting of the seeds associated with the tegument acted as a physical block, impeding the passage of UV light. The results contribute to our understanding of how Persian clover responds to UV radiation exposure. Further investigations should focus on studying the specific impacts of UV radiation on the physiology and biochemical processes of Persian clover seeds, which would provide valuable insights into their response to environmental stressors.

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