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Physical and chemical pretreatments on germination efficiency and seedling growth of *Cannabis sativa*

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ABSTRACT: Seed dormancy in *Cannabis sativa* constrains large-scale cultivation and propagation by limiting uniform and rapid germination. This study evaluated the effects of physical and chemical seed pretreatments, including hot water soaking, nutrient priming, ultrasonication, microwave radiation, and selected conventional methods, on germination performance, seedling vigor, and phytochemical attributes. The methods assessed germination percentage, mean germination time (MGT), seedling length, biomass, seedling vigor index (SVI), total phenolic content (TPC), and antioxidant activity. The results revealed that ultrasonication for 20 min proved the most effective method overall, achieving 86.66% germination, an MGT of 2.84 days, and an SVI of 852.46. Hot water soaking produced the highest germination percentage (93.33%) but a lower SVI (284.32), while nutrient priming with potassium nitrate and calcium chloride resulted in the highest SVI (924.89) despite a longer MGT (4.57 days). Microwave pretreatment (15-20 min) significantly enhanced germination and phytochemical content, with 15 min yielding high antioxidant activity and 73.33% germination. Mechanical scarification, hydrogen peroxide, and dry heat were largely ineffective. Microwave treatment produced the highest TPC (9.22±0.34 mg GAE g^{-1}). These results highlight several effective, scalable pretreatments for improving *C. sativa* propagation and bioactive compound potential.
Keywords: dormancy; seed vigor; emergence; propagation; stress response.

Pré-tratamentos físicos e químicos na eficiência da germinação e no crescimento de mudas de *Cannabis sativa*

RESUMO: A dormência das sementes limita a germinação rápida e uniforme de *Cannabis sativa*, restringindo seu cultivo e propagação em larga escala. Este estudo avaliou os efeitos de pré-tratamentos físicos, químicos e avançados das sementes, incluindo imersão em água quente, priming nutricional, ultrassonicidade, radiação por micro-ondas e métodos convencionais selecionados, sobre o desempenho da germinação, o vigor das plântulas e os atributos fitoquímicos. Foram avaliados a porcentagem de germinação, o tempo médio de germinação (TMG), o comprimento das plântulas, a biomassa, o índice de vigor das plântulas (IVP), o teor de fenólicos totais (TFT) e a atividade antioxidante. A ultrassonicção por 20 min foi o tratamento mais eficaz, com 86,66% de germinação, TMG de 2,84 dias e IVP de 852,46. A imersão em água quente apresentou a maior germinação (93,33%), porém, com menor IVP (284,32), enquanto o priming nutricional com nitrato de potássio e cloreto de cálcio resultou no maior IVP (924,89), apesar de um TMG mais longo (4,57 dias). O tratamento por micro-ondas (15-20 min) melhorou significativamente a germinação e o conteúdo fitoquímico, sendo que 15 min proporcionaram 73,33% de germinação e atividade antioxidante elevada. A escarificação mecânica, o peróxido de hidrogênio e o calor seco foram ineficazes. O maior TFT (9,22 ± 0,34 mg EAG g^{-1}) foi obtido com micro-ondas. Esses resultados destacam pré-tratamentos escaláveis e eficientes para otimizar a propagação de *C. sativa* e seu potencial bioativo.
Palavras-chave: dormência; vigor das sementes; emergência; propagação; resposta ao estresse.

1. INTRODUCTION

Cannabis sativa experiences continuous vegetative growth under long photoperiods and is considered a short-day plant. Once the plant transitions to a shorter photoperiod, inflorescence initiation and development can begin (SALONER et al., 2019). *C. sativa*, known as marijuana or hemp and originating in central Asia, is an annual dioecious flowering plant that belongs to the Cannabaceae family. Male and female sex in the Cannabaceae is determined by heteromorphic chromosomes X and Y (GAUDET et al., 2020; THAWONKIT et al., 2025; THONGPLEW et al., 2025a,b). The gender of the plant is unknown until the flowers grow (SMALL et al., 1976). *Cannabis* is grown for various agricultural uses. Almost all parts of the plant are

used, and seeds are used for food, stem as fiber, leaves and flowers for medicine (CHANDRA et al., 2017). The IUPAC nomenclature of *Cannabis sativa* given by Clarke et al. (2015).

Cannabis is one of the world's oldest crops, dating back some 6000 years. As far as we can discern, there has always been controversy surrounding the use of cannabis. While its narcotic properties often fuel arguments for ongoing criminalization, the potential benefits of cannabis are indisputable. The criminalization of cannabis dates back to the early 20th century, when the global community was concerned about the plant's psychoactive effects as well as the proliferation of international cannabis trade (BEWLEY-TAYLOR et al., 2014). The history of cannabis cultivation remains poorly understood due to the restrictions on scientific enquiry brought about by the drug policy. During the League of Nations' 1925 Geneva Opium Convention, a worldwide foundational framework for the regulation of cannabis was established (SEDDON et al., 2020). After the Marihuana Tax Act of 1937 was passed in the United States, for instance, effectively making cannabis illegal across all states (MUSTO et al., 1972). With a few exceptions, cannabis is still considered illegal in many countries. However, support for its legalization continues to grow. Uruguay becomes the first country to legalize cannabis for its recreational as well as medical use (SEDDON et al., 2020). Possessing a small amount of cannabis is permitted in some countries; however, law enforcement authorities still have the discretion to prosecute in some cases (EASTWOOD et al., 2016). According to Zuk-Golaszewska et al., by 2018, more than half of the US states, as well as Australia, Canada, Germany, South Africa, Uruguay, and other nations, had approved medical cannabis, commonly known as medicinal marijuana.

Germination of Cannabis activates metabolic processes when seeds absorb water. Warmth, moisture, and darkness are essential at this stage. Next, the seed coat cracks open and the taproot emerges, anchoring the plant in the growing medium. This stage of root emergence takes 1-8 days, depending upon conditions and the quality of the seeds; 2-4 days after the root is anchored, two cotyledons (leaves) emerge, marking the start of the seedling stage. Under optimal conditions, which require careful watering to avoid over-saturation, seedlings develop. During the 2-3 week seedling stage, the cannabis plant produces its first true leaves and begins photosynthesis (MISHCHENKO et al., 2017).

Pretreatments help improve germination rates, especially for older or less viable seeds. There are various pretreatments available. One such pretreatment is scarification, which uses various concentrations of sulfuric acid or other chemicals. Another pretreatment involves using sandpaper and a blade (LUERA et al., 2021). The hydration technique involves soaking seeds in normal water instead of hot water (TAYLOR et al.,1992), then treating the immersed seeds with different colors of light, like white light and red light, to check which seeds grow first (OHADI et al., 2010). Other chemical pretreatments involve different chemicals, such as hydrochloric acid or hydrogen peroxide of various concentrations, as well as nutrient solutions like potassium nitrate and sodium chloride (SCHOPFER et al., 2001). To check salinity stress, seeds can be soaked in various concentrations of saline solution (BLANDINIÈRES et al., 2021). A new concept called "osmopriming" is also being used. Seeds are pretreated with osmotic solutions like polyethylene glycol for 12-24 hrs, depending on how old the seed is (VIDAK et al., 2022). There are currently advanced pretreatments available that will soon surpass the traditional pretreatments and will result in improved seed germination. Treatment of seeds with ultrasonic waves and microwaves is one of the best examples of these advanced techniques (ADDO et al., 2022). This study aims to investigate the effect of different pretreatments on the germination rate of *C. sativa* seeds. The experiment will evaluate how various pretreatment methods influence seedling growth and development in *C. sativa*. This study aims to identify the most effective pretreatment technique for promoting optimal seedling health and growth in *C. sativa*.

The objective of the current study is to estimate the effect of temperature, light intensity, and moisture levels as pretreatment factors on *C. sativa* seed germination. Also, this study aims to differentiate the growth performance of seedlings of cannabis exposed to different chemical, mechanical, and environmental pretreatments. The objective is also to detect and estimate key growth parameters like average length of seedling, germination percentage, mean germination time, seedling vigor index, and weight of fresh and dry biomass under each pretreatment condition. To determine the physiological responses of seeds and seedlings of *C. sativa* to various pretreatment methods.

2. MATERIAL AND METHODS

2.1. Sample collection

Seed selection is an important aspect of seed germination. Good quality of seeds will be responsible for high-yielding and disease-free plants. The seeds of *C. sativa* were purchased from a cannabis farm at Maejo University, Chiang Mai, Thailand. The seeds were brought in sealed zip-lock bags for further analysis. The seeds were stored at room temperature (25.0 ± 2.0 °C), avoiding chances of contamination.

2.2. Experimental Setup

This study was conducted in the Pauwadee Laboratory at Maejo University. During this experiment, optimum light conditions were maintained. Every treatment was carried out at room temperature (25.0 ± 2.0 °C). While conducting the study, the relative humidity is maintained between 50 to 70%. Every treatment was carried out in triplicate. In these experiments, a set of controls was performed for each treatment. Control represents those seeds that were not exposed to any of the treatments.

2.3. Seed selection and preparation

2.3.1. Seed quality

Using good-quality seeds is essential for the achievement of the farming system. Factors such as environmental conditions play a significant role in determining seed quality (ELIAS et al., 2020): growing practices during seed development and maturation; harvest methods and post-harvest management are important, including cleaning, drying, and storage. The seeds of *C. sativa* were purchased from a cannabis farm at Maejo University, Chiang Mai, Thailand. The seeds were brought in sealed zip-lock bags for further analysis. The seeds were stored at room temperature (25.0 ± 2.0 °C), avoiding chances of contamination. The seed color plays a major role in seed selection. Dark-colored seeds were associated with maturity, while green-colored seeds were immature, so the dark-colored ones were utilized for the experiments. The seeds were stored for 6 months in the laboratory until the experiments were performed.

2.3.2. Moisture content

Seed moisture is vitally important to control the timing of germination and seedling establishment, especially in species with hard seed coats (JAGANATHAN et al., 2017). In this study for moisture analysis, the AD MX-50 moisture analyzer was used. To analyze this moisture content, it took 10.6 min, and it was done at a temperature of 130 °C with standard mode and low accuracy. To calculate the seed moisture content, equation 01 was used, which was derived from (HAN et al., 2022).

$$\text{SMC} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet wight}} \times 100 \quad (01)$$

2.3.3. Temperature and light

Seed germination is the most sensitive stage in a plant's life cycle, which is an irreversible process that results in the emergence of the radicle and plumule (AMADI et al., 2019). According to Fenner et al. (1991) the effect of light can result in either the death or survival of the embryo. Similarly, a specific temperature is also responsible for germination. To check the temperature and light, a temperature meter (HTC-1 Digital Electronic meter) and a Digital Lux Meter (SMART SENSOR AS803) were used, respectively.

2.3.4. Surface sterilization of seeds

Contamination with microbes is considered the reason for losses during seed germination. Maintaining the integrity of the material while at the same time ensuring it is free from harmful contaminants (Oyebanji et al., 2009) is an important balance to maintain. Seeds were sterilized on their outer surface by using disinfectants such as ethanol and sterile water to remove possible contaminants (LINDSEY et al., 2017).

2.4. Physical pretreatment methods

2.4.1. Scarification

Chemical scarification involves using basic or acidic solutions to soften the seed coating. This type of scarification enables germination by allowing oxygen and water to penetrate the coating. This method mimics natural processes like digestion in animals or environmental factors. Similarly, seeds (15 no.) were introduced to different chemicals like hydrochloric acid, phosphoric acid (25%-75%), and hydrogen peroxide (3%) for different time intervals like 0, 5, 10, 15, and 20 min. At the conclusion of each time interval, we removed excess acid residue, and then the treated seeds were washed with sterile distilled water. These seeds were then placed on a wet kitchen towel in a tray for germination. We recorded the results at specific time intervals (KIMURA et al., 2012). Each treatment was performed in triplicate.

Mechanical scarification is a method used to break seed dormancy by physically altering the seed coat to allow water and air penetration, promoting germination. In this experiment, 15 seeds (15 no.) were placed between the two sheets of sandpaper with a grit of 100 to 150. After several passes, the seeds were examined to ensure that a sufficient portion of the outer shell had been removed, which would allow the moisture to enter inside. Then, after successful scarification, these seeds were soaked in sterile distilled water in a zip-lock bag for germination, and results were noted (GONG et al., 2024). These treatments were performed in triplicate.

Heat scarification is a method used to enhance seed germination by weakening hard seed coats through controlled thermal exposure. This technique mimics natural processes like wildfires or digestive heat in animals, which break down the seed dormancy mechanism. In this experiment, 15 seeds (15 no.) were introduced to dry heat in a hot air oven for specific time intervals, like 0, 5, 10, 15, and 20 min. Then these seeds were soaked in sterile distilled water for 24 hours, and then on a wet kitchen towel for germination. Results were recorded at a specific time (GONG et al., 2024). These treatments were performed in triplicate.

2.4.2. Hydration Techniques

Hot water can physically weaken seed coats or induce physiological changes to overcome dormancy. In this study15 seeds are soaked in hot sterile distilled water for 24 hrs in a sterile glass bottle. After that, place on a wet (not moist) kitchen towel in a tray for germination. After 24 hours, the results were noted (MOHSENKHAH et al., 2018). These treatments were performed in triplicate. Cold water softens seed coats and initiates metabolic processes. Fifteen seeds are soaked in cold/ normal sterile distilled water for 24 hrs in a sterile glass bottle. After that, place on a moist (not wet) kitchen towel in a tray for germination. After 24 hours, results were noted (MOHSENKHAH et al., 2018). These treatments were performed in triplicate.

2.4.3. Light Exposure

Light and duration of exposure to light are essential factors in the seed germination process. The intensity and duration of light exposure can influence the timing and success of seed germination in these species. In this experiment, the seeds were first soaked in hot water for about 24 hours. Then these seeds were sown on a wet kitchen towel and exposed to white light and red light of the same frequency, and then kept for germination for a specific period. Results were recorded at specific time intervals (JIN et al., 2021). These treatments were performed in triplicate.

2.5. Chemical Pretreatments

2.5.1. Acid Pretreatment

Sulfuric acid (H_2SO_4) is widely used to overcome seed coat dormancy in hard-seeded species by physically weakening the seed coat, enabling water absorption and gas exchange. In this study, seeds were introduced to sulfuric acid with different concentrations, like 15%, 50%, 80%, and 96%, for a time interval of 60 min. Then, after 60 minutes, the seeds were washed with sterile distilled water multiple times to remove traces of acid from their surface. After washing, these seeds for germination were sown on a tray covered by a wet kitchen towel. Results were recorded at specific time intervals (ŠOCH et al., 2023). These treatments were performed in triplicate.

2.5.2. Nutrient Soaking

Soaking seeds in nutrient solutions can enhance germination by softening the seed coat and providing essential nutrients for early growth. 15 seeds were soaked in a solution of 0.5 % potassium nitrate for 24 hours and then again soaked in a calcium chloride solution of 0.2 % concentration for another 24 hours. After soaking, the seeds were sown on a wet kitchen towel in a tray for germination. Results were recorded at specific time intervals. These treatments were performed in triplicate. Potassium nitrate provided nitrogen and potassium, which are essential for

protein synthesis, energy transfer, and root development. This nutrient solution also helps to overcome dormancy by promoting the activation of the enzyme. At the same time, calcium chloride is essential for cell wall stabilization and membrane integrity. It softens the seed coat, allowing water to penetrate (KLAROD et al., 2021).

2.5.3. Salinity Stress Testing

The effects of saline solutions on seed germination are determined by osmotic stress, ion toxicity, and species-specific tolerance mechanisms. Saline solutions reduce seed imbibition rates by delaying germination. Seeds were treated with a mild saline solution of 0.9% concentration prepared by dissolving sodium chloride in distilled water (HMISSI et al., 2023). After treatment for 24 hours, the soaked seeds were sown on a wet kitchen towel for germination. Results were recorded at specific time intervals. These treatments were performed in triplicate.

2.6. Osmopriming

The 15 seeds were soaked in a prepared PEG solution of 15 % concentration for about 24 hours. Then, after soaking, these seeds were thoroughly washed with distilled water several times to remove excess solution. After washing, seeds were sown on a wet kitchen towel for germination, and results were recorded at different time intervals (MA et al., 2024). These treatments were performed in the triplicates. Primed seeds exhibited better osmotic regulation and antioxidant capacity under drought or salt stress. While mild PEG concentrations (e.g., 10%) improve germination, higher concentrations (e.g., 30%) reduce vigor and seedling mass (MAHPARA et al., 2022).

2.7. Advanced pretreatment methods

2.7.1 Microwave radiation

In this experiment, 15 seeds were exposed to microwave radiation at different time intervals, such as 5, 10, 15, and 20 minutes. Then these seeds were sown in a zip-lock bag containing a moist kitchen towel. Then these bags were kept in periodic light and dark conditions for germination. Results were recorded at specific time intervals (MOHSENKHAH et al., 2018). These treatments were performed in triplicate.

2.7.2. Ultrasonication

In this experiment, 15 seeds were exposed to ultrasonic waves for different time intervals, like 10, 20, 30, and 40 minutes, maintaining the temperature in an Ependorf tube submerged fully in the sonication tank filled with water. After sonication, these seeds are again soaked in fresh sterile distilled water for 24 hours. Then the seeds were sown on a wet kitchen towel in a tray for germination. Results were recorded at specific time intervals (GONG et al., 2024). These treatments were performed in triplicate.

2.7.3. Germination and growth assessment

Seeds with a hard seed coat require pretreatments to break dormancy. Soaking in warm water and then scratching the seed coat can reduce germination time by allowing water and oxygen to penetrate the seed coat. The seeds were allowed to germinate on the wet kitchen towel. According to Frassinetti et al. (2018), once the sprout germinated for 4 to 7 days, the germination process was stopped.

The length of the sprout depends upon water, oxygen, and nutrients present in the endosperm. Once germination begins, radical emergence and shoot elongation occur under favorable conditions. To measure the length of the sprout vernier caliper was used. To calculate the average length of the sprout, Equation 02 was used;

$$\text{Avg . Lenght of Sprout} = \frac{\text{Lenth of each sprout (mm)}}{\text{No .of seeds sprouted}} \qquad (02)$$

Pretreatment methods can enhance the germination rate, without which the percentage of germination (%) remains low due to the impermeability of the seed coat. Equation 03 was adopted from Božena (2023). To calculate the percentage of germination or germination emergence, the formula used was

$$\text{GP} = \frac{\text{no.of seeds germinated}}{\text{total no of seeds}} \times 100 \qquad (03)$$

Mean germination time (MGT) is influenced by pretreatment methods and environmental conditions such as temperature and moisture. For seeds with a hard seed coat, MGT decreases when dormancy-breaking techniques are applied. To determine the mean germination time, no. of seeds that germinated on each day is required. The formula (4) was adopted from Irik et al. (2024).

$$\text{MGT} = \frac{\text{No of seeds germinated} \times \text{day of germination}}{\text{total no of seeds germinated}} \qquad (04)$$

Seedling vigor index (SVI) is determined by the rapidity and uniformity of germination and early growth stages. High vigor is seen when seeds are treated properly to ensure uniform water uptake and metabolic activation. To determine seedling vigor, the growth length of the seedling and the germination percentage are required. The formula (5) was adopted from (PRADEEP et al., 2018). So to calculate SVI, the equation used was;

$$\text{SVI} = \text{SL} \times \text{GP}\,\% \qquad (05)$$

where: SVI= seedling vigor index; SL= seedling length; GP = germination percentage.

Biomass accumulation depends on nutrient availability and photosynthesis post-germination. Treated seeds often show higher biomass due to better initial growth conditions. To calculate the fresh as well as dry biomass of seedlings, an analytical balance was used, and the results were noted in grams.

2.8. Microscopic analysis

2.8.1. Microscopic camera

To get the magnified image of the sprout, a 3-in-1 camera microscope (model AN104) was used. It is a digital microscope used for observing small objects in detail. The microscope offers magnification up to 1000x or 1600x and has an adjustable LED light for clear imaging. The camera was connected to a computer. The magnified pictures of sprouts were captured using an application for cameras.

2.8.2. Phytochemical analysis

Phytochemical analysis involves the detection, characterization, and quantification of bioactive compounds in plants, which are critical for understanding their medicinal and industrial applications. Qualitative tests identify specific phytochemical classes using chemical reactions and visual

observations. (VELAVAN et al.,2015). Some advanced quantitative analysis techniques identify the concentration of phytochemicals.

2.9. Extraction of bioactive

The test sample was powdered using a mortar and pestle. The powder was subjected to extraction using hexane. Dried and powdered sprouts were subjected to Soxhlet extraction. Approximately 2 grams of the dried sprout powder was placed inside a Whatman cellulose extraction thimble (33 × 80 mm), which was inserted into the main chamber of a Soxhlet extractor. Hexane (analytical grade, 100 mL) was used as the solvent due to its non-polar nature, which effectively extracts lipophilic compounds such as cannabinoids, terpenes, and essential oils. The round-bottom flask containing the hexane was placed in a heating mantle and maintained at the boiling point of hexane (approx. 68°C) throughout the process. A reflux condenser was attached to the top of the extractor, and continuous water flow was maintained through the condenser to ensure proper condensation of hexane vapors. As the solvent vaporized, it condensed and dripped into the thimble containing the plant material. Once the chamber filled up to the siphon level, the solvent, along with extracted compounds, was siphoned back into the boiling flask. This reflux-siphon cycle allowed repeated washing of the plant material with fresh solvent. The extraction was carried out for 6 hours. After the extraction process was complete, the hexane extract in the flask was transferred to a petri plate to remove the solvent, yielding a semi-solid crude extract. The crude extract was then stored at 4°C in a vial for further analysis.

2.9.1. Total Phenolic Content (TPC)

The TPC analysis method was modified from the previously described method by Behbahani et al. (2017). The estimation of total phenols by the FCR (Folin-Ciocalteu Reagent) method of the test sample was carried out. 1 mL of test sample and standard (Gallic acid) was made of different concentrations (300, 600, and 900 μg mL^{-1}) in beakers, followed by 1 mL of FC reagent and incubation of the beakers for 5 min. Then, 10 mL of 7% sodium carbonate and 25 mL of distilled water were added to the beaker and incubated for 90 min. Absorbance is measured at 560 nm (BEHBAHANI et al., 2017).

2.9.2. Radical scavenging assay (DPPH method)

Antioxidant activity is analyzed by modifying the previously stated method by Yeganagi (2018) et al. Antioxidant activity of the test sample was estimated for its free radical scavenging activity by using the DPPH method. 1 mL of different concentrations (20, 40, 60, 80, and 100 μg mL^{-1}) was taken in test tubes. Then 1.5 mL of 0.1% methanolic DPPH was added to the sample and incubated for 30 min in dark conditions. The sample was observed for discoloration from purple to yellow, and the absorbance at 530 nm. Radical scavenging activity was calculated by using equation 06 suggested by Yeganegi et al. (2018).

$$\text{DPPH} = \frac{\text{OD C} - \text{OD TS}}{\text{OD C}} \times 100 \qquad (6)$$

where: DPPH = radical scavenging activity; C = control; TS = test sample; OD = optical density.

2.10. Statistical Analysis

Each experiment had a total of 15 seeds, and all the experiments were conducted in triplicate. Descriptive statistics, including mean and standard deviation, were calculated for each treatment group. One-way ANOVA was used to assess significant differences in germination percentage, mean germination time (MGT), seedling vigor index (SVI), and biomass across pretreatment methods. A significance level of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Seed selection and preparation

3.1.1. Seed quality

According to Horakova et al. (2020), ethanol was diluted to 70 % and samples were soaked in it for 30 min, then rinsed twice with PBS and distilled water. Finally, samples were allowed to dry for further testing. Similarly, brown colored mature seeds were selected and then subjected to surface sterilization, in which they were treated with 70% ethanol for 3 minutes and then washed with sterile distilled water 3 times to remove traces of ethanol. Each experiment had a total of 15 seeds in triplicate, and the seeds were selected for further experiments.

3.1.2. Moisture content

In this study, the moisture content of 0.1 g of seeds turned out to be 18.61. In seeds of adzuki bean (*Vigna angularis*), the seed coat was permeable at 29.94% moisture content (dry basis) and became impermeable when the moisture content was lowered to 14.96% (MIANO et al., 2015).

3.1.3. Temperature and light

Temperature is a crucial factor in the germination of seeds. It significantly influences the rate and speed of germination. Temperatures above or below the optimal range may harm the germination process. High temperature leads to heat stress, causing seed damage, while low temperature can slow down the metabolic process, causing delayed germination. That's why the entire experiment was carried out at room temperature, that is, 25.0 ± 2.0 °C. Light intensity is maintained at 6500 lux (SGHAIER et al., 2022).

3.2. Germination and growth assessment

The germination and growth assessment is done with several of the above-mentioned methods, and the obtained results are provided in the sections below. For germination counting, a seedling was considered morphologically normal when it showed complete and healthy development of essential seedling structures, following standard seed testing guidelines. Specifically, a seed was recorded as germinated when the radicle had visibly emerged (≥ 2 mm) and continued to elongate, accompanied by normal hypocotyl growth and the symmetrical emergence of intact cotyledons. Normal seedlings exhibited a straight, well-developed radicle without necrosis, swelling, or breakage; a firm and uninjured hypocotyl; and green, undamaged cotyledons capable of supporting early growth. Seedlings showing abnormalities such as split or decayed radicles, stunted or malformed hypocotyls, missing or severely damaged cotyledons, fungal infection, or arrested growth were classified as abnormal and excluded from germination counts.

3.3. Physical pretreatment methods

3.3.1. Chemical scarification

1% H_2O_2 solution was used on the seeds for germination. But in this case, the germination was not successful. Several factors may have contributed to the failure of germination under H_2O_2 treatment. One possibility is that seeds possess a relatively hard seed coat, and H_2O_2 alone may have been insufficient to overcome the physical dormancy. It is also possible that the seeds used in the experiment were already compromised, which may have further contributed to the lack of response. Moreover, the concentration or exposure duration used in the experiment might have been too high or too low, which would have led to oxidative stress and damage to embryonic tissue. The results suggest that while H_2O_2 treatment is useful for some species, its application in *C. sativa* requires careful optimization of concentration, soaking duration, and potentially a combination with other physical and chemical pretreatments to get the desired results.

3.3.2. Mechanical scarification

The sandpaper treatment was the most appropriate dormancy-breaking method for A. sikokianus seeds that had a hard seed coat (JIN et al., 2023). Despite the potential of this method in promoting germination in hard-seeded species, the results (Figure 1) indicated no germination in seeds subjected to sandpaper scarification. This outcome suggests that the degree of abrasion applied may have been either insufficient to break the dormancy or, conversely, too aggressive, possibly damaging the embryo. Therefore, while sandpaper scarification is generally effective in species with hard, water-impermeable seed coats, its effectiveness in *C. sativa* may be limited unless optimized in combination with other dormancy-breaking treatments.

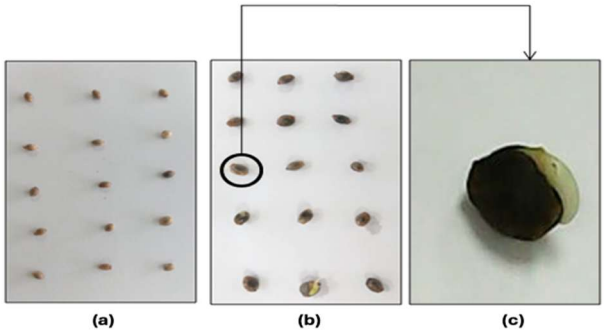

Figure 1. Seeds were exposed to dry heat in an oven for a specific time at a controlled temperature and then were sown on a wet kitchen towel for germination (a). Results after 7 days of sowing (b). Magnified view of sprout (c).
Figura 1. Sementes foram expostas ao calor seco em uma estufa por um tempo específico, em temperatura controlada, e então semeadas em um pano de prato úmido para germinação (a). Resultados após 7 dias da semeadura (b). Visão ampliada do broto (c).

3.3.3. Thermal/ Heat scarification

Thermal scarification by using oven heat was not successful. The exceptionally low germination rate of 3.33 % implies excessive damage or inappropriate temperature or time during heat exposure, as presented in Table 1. The seedling that manages to grow has a reasonable sprout length, but the overall success was poor.

Thermal or dry heat scarification has been successfully employed to overcome water impermeability in various legume species. The application of heat induces microcracks in the seed coating and splits the palisade layer of strophiole, thereby accelerating the imbibition process and facilitating quicker germination (BASKIN; BASKIN, 1998). It has been one of the most popular methods because of its simple and easy use (HARRINGTON, 1916). Agricultural value of impermeable seeds) and a hot water bath (Uzun; Aydin 2004), can be used for thermal scarification.

Table 1. Effect of thermal and heat time on sprouting and seedling growth parameters of the *C. sativa* seeds.
Tabela 1. Efeito do tempo térmico e do calor sobre os parâmetros de brotação e crescimento de mudas de sementes de *C. sativa*.

Parameters	Results
Number of seeds tested	15
Germinated seeds	3
Time (days)	7
Average length of sprout (mm)	11.04
Wet biomass (gm)	0.30
Dry biomass (gm)	0.04-
Germination percent (%)	20
Mean germination time MGT (days)	3.33
Seedling vigor index SVI	287.6

3.3.4. Hydration Techniques

Hot water treatment is an effective way to break seed dormancy. In the present experiment, this process increased the seed germination percentage up to 93.33 %. The sprouts were also much longer and healthier (eg, Seed 5 with 8.53 mm length h) (Figure 2). The germination was also faster, with a mean germination time of 3.42 days, showing hot water effectively weakened the seed coat with an SVI of 284.32 (Table 2).

Figure 2. Germination was observed by hot water soaking.
Figura 2. A germinação foi observada por meio de imersão em água quente.

Table 2. Effect of hot water soaking on sprouting and seedling growth parameters of the *C. sativa* seeds.
Tabela 2. Efeito da imersão em água quente sobre os parâmetros de brotação e crescimento de mudas de sementes de *C. sativa*.

Parameters	Results
Number of seeds tested	15
Germinated seeds	14
Time (days)	7
Average length of sprout (mm)	3.55
Wet biomass (gm)	1.43
Dry biomass (gm)	0.20
Germination percent (%)	93.33
Mean germination time MGT (days)	3.42
Seedling vigor index SVI	284.32

Seeds treated with normal water did not produce much yield. Only a few seeds produced sprouts, with modest sprout length observed (Figure 3). Normal water soaking allows for imbibition but does not provide any physical or chemical stimulus to disrupt the seed coat structure or activate physiological processes. As a result, germination percentage

tends to be lower, and mean germination time (MGT) is higher compared to seeds subjected to scarification or thermal pretreatments (ALIERO, 2004). In the case of *C. sativa*, although seeds do not possess an extremely hard seed coat, certain batches may exhibit dormancy due to incomplete after-ripening, poor moisture content, or storage-related physiological dormancy (SCHLUTTENHOFER; YUAN, 2017). In such cases, normal water soaking fails to break dormancy effectively, resulting in erratic or delayed germination. The longest seedling length recorded was seed 10 with 4.10 mm. The germination percentage was also very low at 26.66 %. The mean germination time recorded was 3 days with 81.77 SVI. Wet (0.262 gm) and dry (0.038 gm) biomass accumulation was also much lower than that presented in Table 3. Normal water treatment possibly slowed down the metabolic activity necessary for quicker and higher germination. As the seed coat becomes impermeable to water, the hilar fissure of the seed acts like a valve that allows water vapor to diffuse out of the seed under low surrounding relative humidity, and it prevents water from entering the seed under high humidity (HYDE et al., 1954).

Figure 3. Germination was observed by normal water soaking.
Figura 3. Germinação observada por imersão em água normal.

Table 3. Effect of normal water soaking time on sprouting and seedling growth parameters of the *C. sativa* seeds.
Tabela 3. Efeito do tempo normal de imersão em água sobre os parâmetros de brotação e crescimento de mudas de *C. sativa*.

Parameters	Results
Number of seeds tested	15
Germinated seeds	4
Time (days)	7
Average length of sprout (mm)	3.07
Wet biomass (gm)	0.26
Dry biomass (gm)	0.04
Germination percent (%)	26.67
Mean germination time MGT (days)	3
Seedling vigor index SVI	81.77

3.3.5. Light exposure

In this experiment, white light exposure supported a moderate level of seed germination (Figure 4). Germination percentage (60 %) and mean germination time of 3.33 days with SVI of 4.12 were obtained, indicating that though germination occurred, the strength and robustness of the seedling remained weak. The amount of wet (0.23 g) and dry (0.032 g) biomass obtained was also quite low. Although some seeds achieved a good length of sprout with seed 2 (7.32 mm) and seed 3 (7.90 mm), as presented in Table 4.

In the present study, exposure to white light resulted in a moderate level of seed germination, indicating that light may play a regulatory role in the germination process, although it was not the most dominant factor in enhancing germination percentage or seedling vigor. These results are consistent with the general understanding that light acts as an important environmental cue, especially for photoblastic seeds, those that require or are inhibited by light to germinate (BASKIN; BASKIN, 2000).

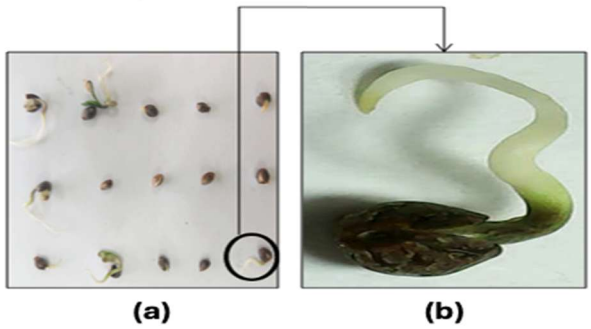

Figure 4. Seeds were sown on a wet kitchen towel and exposed to white light, and allowed to germinate. Results after 7 days of sowing (a). Magnified view of sprout (b).
Figura 4. As sementes foram semeadas em um pano de prato úmido e expostas à luz branca para germinar. Resultados após 7 dias da semeadura (a). Visão ampliada do broto (b).

Table 4. Effect of white light exposure time on sprouting and seedling growth parameters of the *C. sativa* seeds.
Tabela 4. Efeito do tempo de exposição à luz branca sobre os parâmetros de brotação e crescimento de mudas de sementes de *C. sativa*.

Parameters	Results
Number of seed tested	15
Germinated seeds	9
Time (days)	7
Average length of sprout (mm)	4.22
Wet biomass (gm)	0.23
Dry biomass (gm)	0.03
Germination percent (%)	60
Mean germination time MGT (days)	3.33
Seedling vigor index SVI	4.21

Exposure to red light alone was insufficient for seed germination. It resulted in a low germination percentage (26.66 %) and extremely weak seedlings (Figure 5). Seed 13 with a 2.70 mm length was the highest amongst all germinated seeds. Most other seeds did not germinate under red light. Still, with a rapid mean germination time of 2 days, the overall seedling vigor index remains low (1.48) as presented in Table 5. Red light primarily activates phytochrome B, a light-sensitive photoreceptor that regulates the transition of the phytochrome system from its inactive to its active form. This activation is generally associated with the promotion of germination in many photoblastic seeds. The activation leads to the upregulation of genes responsible for gibberellin biosynthesis and the breakdown of abscisic acid, the primary hormone responsible for maintaining dormancy (KAMI et al., 2010).

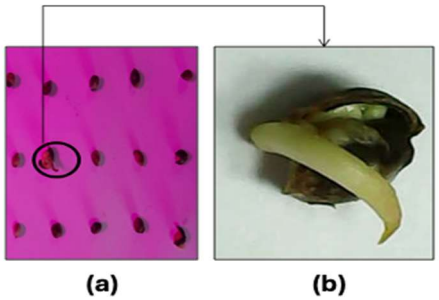

Figure 5. Seeds were sown on a wet kitchen towel and exposed to red light, and allowed to germinate. Results after 7 days of sowing (a). Magnified view of a sprout (b).

Figura 5. As sementes foram semeadas em um pano de prato úmido e expostas à luz vermelha, e deixadas para germinar. Resultados após 7 dias da semeadura (a). Visão ampliada do broto (b) .

Table 5. Effect of red light exposure time on sprouting and seedling growth parameters of the *C. sativa* seeds.

Tabela 5. Efeito do tempo de exposição à luz vermelha sobre os parâmetros de brotação e crescimento de mudas de sementes de *C. sativa*.

Parameters	Results
Number of seeds tested	15
Germinated seeds	4
Time (days)	7
Average length of sprout (mm)	1.48
Wet biomass (gm)	0.02
Dry biomass (gm)	0.01
Germination percent (%)	26.67
Mean germination time MGT (days)	2
Seedling vigor index SVI	1.48

3.4. Chemical Pretreatments

3.4.1. Acid Pretreatment

Acid treatment can effectively break down seed coat dormancy, but it depends on the concentration of acid. Low concentrations (15 %) were too mild to break down the seed coat, where two seeds managed to sprout (seeds 1 with 1.12 mm length and seed 3 with 1.02 mm length) with a mean germination time of 4 days and 14.26 of SVI. Moderate concentrations like 50 % can improve water uptake, where 4 seeds manage to germinate (eg, seed 6 with 2.09 mm length) with 3 days of mean germination time and 32.79 of SVI. Then, 80 % concentrated acid gave improved germination with 2.25 days of mean germination time and an increased SVI of 49.12. Here, the length of the observed sprout was also 2.99 mm, which is the highest among all germinated seeds. Lastly, higher concentrations like 96 % caused severe damage or death of seeds due to acid burns, hence no germination was observed. Collectively, wet (1.69 g) and dry biomass (0.236 g) were obtained (Table 6).

Table 6. Effect of acid treatment time on sprouting and seedling growth parameters of the *C. sativa* seeds.

Tabela 6. Efeito do tempo de tratamento com ácido sobre os parâmetros de brotação e crescimento de mudas de *C. sativa*.

Parameters	H_2SO_4 (%)			
	15	50	80	90
Number of seeds tested	15	15	15	15
Germinated seeds	2	4	4	0
Time (days)	7	7	7	7
Average length of sprout (mm)	1.07	1.23	1.84	-
Wet biomass (gm)	1.69	3.38	3.24	-
Dry biomass (gm)	0.24	0.48	0.39	-
Germination percent (%)	13.33	26.66	26.66	-
Mean germination time (days)	4	3	2	-
Seedling vigor index	14.26	32.79	49.12	-

3.4.2. Nutrient Soaking

The nutrient priming has considerably improved germination capacity and seedling vigor. Delayed but enhanced mean germination time (4.57 days) and increased seedling SVI (924.89) were observed (Figure 6). Large sprout length (eg, Seed 15 with 41.01 mm length) and high germination percentage (73.33 %) suggest that treatment with nutrient solutions has prepared the seeds for better uptake of water and metabolic activation. Overall wet biomass (0.159 gm) and dry biomass (0.034 gm) accumulation reflected healthier physiological performance as presented in Table 7.

3.4.3. Salinity Stress Testing

Saline stress has a significant role in seed germination and early seedling development. Exposure to saline solution for 24 hours affected nearly half of the seeds, which failed to germinate, and among those that germinated, there was a difference in length. Some seeds managed good sprouting (eg, Seed 8 with 12.39 mm length), suggesting partial tolerance to salinity (Figure 7). Wet biomass (0.120 gm) indicates the mass of the fresh seedlings immediately after germination, whereas dry biomass (0.016 gm) gives the mass after drying the seedlings. The overall germination percentage of seedlings was 46.66 %, which indicates reduced viability due to saline stress. The average time for seeds to germinate was 2.85 days, showing rapid germination among the seeds. A higher SVI 452.80 suggests better performance despite stress conditions, as presented in Table 8.

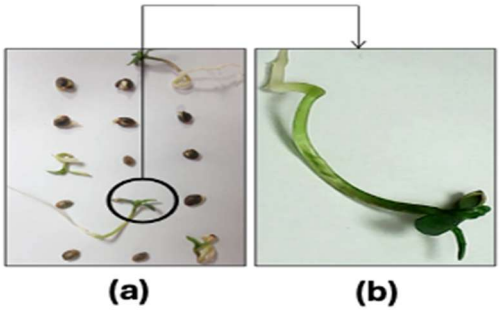

Figure 6. Seeds were treated with nutrient solutions and sown on a wet kitchen towel for germination. Results after 7 days of soaking (a). Magnified view of a sprout (b).

Figura 6. As sementes foram tratadas com soluções nutritivas e semeadas em um pano de prato úmido para germinação. Resultados após 7 dias de imersão (a). Visão ampliada do broto (b).

Table 7. Effect of nutrient soaking time on sprouting and seedling growth parameters of the *C. sativa* seeds.

Tabela 7. Efeito do tempo de imersão de nutrientes sobre os parâmetros de brotação e crescimento de mudas de sementes de *C. sativa*.

Parameters	Results
Number of seeds tested	15
Germinated seeds	11
Time (days)	7
Average length of sprout (mm)	12.61
Wet biomass (gm)	1.59
Dry biomass (gm)	0.34
Germination percent (%)	73.33
Mean germination time MGT (days)	4.57
Seedling vigor index SVI	924.89

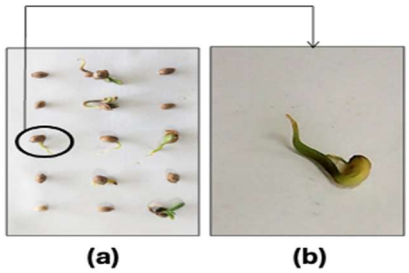

Figure 7. Seeds were treated with saline and sown on a wet kitchen towel for germination. Results after 7 days of soaking (a). Magnified view of a sprout (b).

Figura 7. As sementes foram tratadas com solução salina e semeadas em papel-toalha úmido para germinação. Resultados após 7 dias de imersão (a). Visão ampliada do broto (b).

Table 8. Effect of salinity stress time on sprouting and seedling growth parameters of the *C. sativa* seeds.
Tabela 8. Efeito do tempo de estresse salino sobre os parâmetros de brotação e crescimento de mudas de *C. sativa*.

Parameters	Results
Number of seeds tested	15
Germinated seeds	7
Time (days)	7
Average length of sprout (mm)	9.70
Wet biomass (gm)	0.12
Dry biomass (gm)	0.07
Germination percent (%)	46.66
Mean germination time MGT (days)	2.85
Seedling vigor index SVI	452.80

3.4.4. Osmopriming

The application of the PEG solution for 24 hours created osmotic stress conditions. Only a small number of seeds could germinate under this treatment (Figure 9). Seeds that managed to germinate (eg, Seed 7 with 17.55 mm length) showed significantly longer sprout length, indicating partial tolerance. A lower germination percentage (33.33 %) and reduced SVI (235.37) suggest that PEG-induced stress imposes rigorous limitations on water uptake essential for germination. Here, the wet biomass (0.18 g) and dry biomass (0.025 g) were relatively low, as illustrated in Table 9.

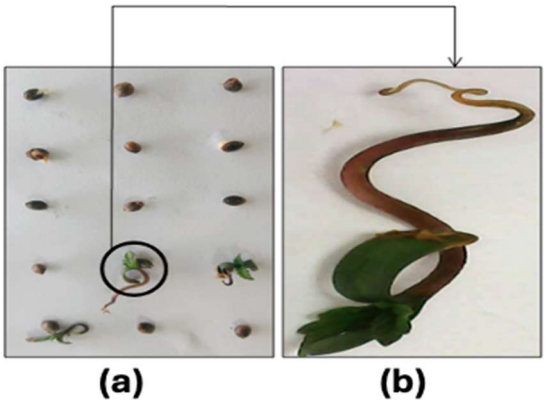

Figure 9. Seeds were treated with PEG and sown on a wet kitchen towel. Results after 7 days of sowing (a). Magnified view of sprout (b).
Figura 9. As sementes foram tratadas com PEG e semeadas em papel-toalha úmido. Resultados após 7 dias da semeadura (a). Visão ampliada do broto (b).

3.5. Advanced pretreatment methods

3.5.1. Microwave radiation

A 15-minute microwave exposure showed the highest germination rate (73.33%), indicating improved dormancy breaking (Figure 10). Control and 5-minute treatments showed moderate germination (~53.33%). Slight reduction in germination at 10 min (46.66%). 10 min exposure resulted in the longest sprouts (~40 mm). However, 20-minute and 15-minute treatments had higher SVI values due to their combination of good sprout length and high germination %. SVI peaked at 20 min, suggesting optimal vigor as illustrated in Table 10. The shortest germination time was observed in 5–10 min groups. Microwave pretreatment enhances seed germination and seedling vigor in *C. sativa*. 15–20 minutes of exposure yields the most promising results, with a balance of high germination % and sprout growth. Excessive or insufficient exposure may reduce effectiveness.

Table 9. Effect of osmopriming time on sprouting and seedling growth parameters of the *C. sativa* seeds.
Tabela 9. Efeito do tempo de osmocondicionamento sobre os parâmetros de brotação e crescimento de mudas de sementes de *C. sativa*.

Parameters	Results
Number of seed tested	15
Germinated seeds	5
Time (days)	7
Average length of sprout (mm)	8.36
Wet biomass (gm)	0.18
Dry biomass (gm)	0.03
Germination percent (%)	33.33
Mean germination time MGT (days)	2
Seedling vigor index SVI	235.37

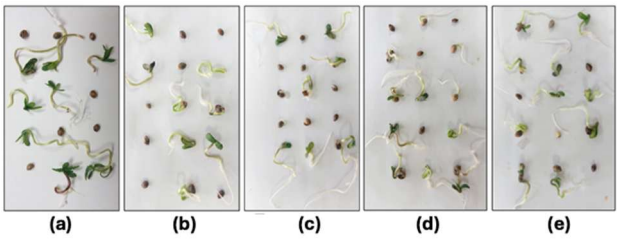

Figure 10. Seeds were treated with microwave radiation for (a) 0 min, (b) 5 min, (c) 10 min, (d) 15 min, (e) 20 min, and sowed on a wet kitchen towel. Results were observed after 7 days of sowing.
Figura 10. As sementes foram tratadas com radiação de micro-ondas por (a) 0 min, (b) 5 min, (c) 10 min, (d) 15 min, (e) 20 min e semeadas em um pano de prato úmido. Resultados após 7 dias da semeadura.

Table 10. Effect of microwave time on sprouting and seedling growth parameters of the *C. sativa* seeds.
Tabela 10. Efeito do tempo de micro-ondas sobre os parâmetros de germinação e de crescimento de mudas de sementes de *C. sativa*.

Parameters	Microwave Time (min)				
	0	5	10	15	20
Number of seed tested	15	15	15	15	15
Germinated seeds	8	8	7	11	10
Time (days)	7	7	7	7	7
Average length of sprout (mm)	31.46	29.43	40.25	26.63	27.61
Wet biomass (gm)	4.1	3.83	5.24	3.47	3.60
Dry biomass (gm)	0.56	0.52	0.71	0.47	0.49
Germination percent (%)	53.33	53.33	46.66	73.33	66.66
Mean germination time MGT (days)	3.37	3.37	3.85	3.72	3.90
Seedling vigor index SVI	167.89	156.86	187.59	195.23	184.03

3.5.2. Ultrasonication

Treatment of ultrasonic waves for different time intervals was performed to evaluate their effect on seed dormancy break and early seedling vigor: 0, 10, 20, 30, and 40 min. Ultrasonication at moderate levels enhances seed germination by weakening the seed coat, improving water

imbibition, and potentially activating enzymes. The 20-minute treatment was optimal for *C. sativa*, significantly increasing germination and vigor (Figure 11). However, prolonged exposure (40 min) likely damaged seed structure or disrupted physiological processes, reducing performance. Table 11 summarizes the effect of ultrasonication time on seed germination and early seedling growth. Overall, moderate ultrasonication (20–30 min) markedly enhanced germination performance compared with the control (0 min) and longer duration exposure. The 20-minute treatment showed the best overall response, with the highest germination percentage (86.66%), the shortest mean germination time (2.84 days), and the maximum seedling vigor index (SVI = 852.46), indicating faster and more vigorous seedling establishment. The 30-minute treatment also improved seedling length and vigor, but resulted in a lower germination percentage (60%). In contrast, prolonged ultrasonication (40 min) severely reduced germination (13.33%), increased MGT, and resulted in negligible seedling growth, suggesting cellular or physiological damage. The control and 10-minute treatments showed moderate responses, confirming that ultrasonication has a dose-dependent effect, with optimal exposure enhancing germination and excessive exposure being detrimental.

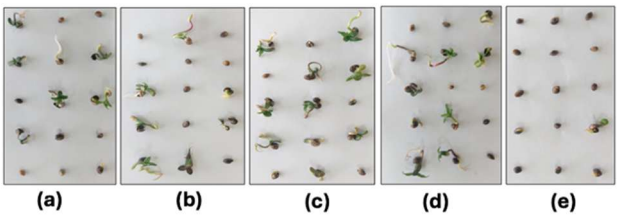

Figure 11. Seeds were treated with ultrasonic waves for (a) 0 min, (b) 10 min, (c) 20 min, (d) 30 min, (e) 40 min, and sown on a wet kitchen towel. Results after 7 days of sowing.
Figura 11. As sementes foram tratadas com ondas ultrassônicas por (a) 0 min, (b) 10 min, (c) 20 min, (d) 30 min, (e) 40 min e semeadas em um pano de prato úmido. Resultados após 7 dias da semeadura.

Table 11. Effect of ultrasonication time on sprouting and seedling growth parameters of the *C. sativa* seeds.
Tabela 11. Efeito do tempo de ultrassonicção sobre os parâmetros de brotação e crescimento de mudas de *C. sativa.*

Parameters	Ultrasonication time (min)				
	0	10	20	30	40
Number of seeds tested	15	15	15	15	15
Germinated seeds	7	7	13	9	2
Time (days)	7	7	7	7	7
Average length of sprout (mm)	9.28	10.50	10.77	13.79	-
Wet biomass (gm)	2.51	3.44	3.53	4.51	-
Dry biomass (gm)	0.35	0.48	0.49	0.63	-
Germination percent (%)	46.66	46.66	86.66	60	13.33
Mean germination time MGT (days)	2.14	2.28	2.84	2.66	5
Seedling vigor index SVI	433.13	489.93	933.68	827.40	-

The results illustrated in Figure 14 demonstrate that among the various physical, chemical, and advanced pretreatment methods tested, ultrasonication for 20 minutes emerged as the most effective technique for enhancing seed germination and seedling vigor in *C. sativa*. This method yielded a high germination percentage of 86.66%, with a mean germination time (MGT) of 2.84 days, and an impressive seedling vigor index (SVI) of 852.46. The treatment facilitated better water absorption and activated metabolic processes by creating micro-channels in the seed coat through acoustic cavitation. In comparison, hot water soaking, a traditional and cost-effective approach, produced the highest germination rate of 93.33%, though with a relatively lower SVI (284.32), indicating less vigorous seedlings. On the other hand, nutrient soaking with potassium nitrate and calcium chloride led to the highest SVI (924.89), suggesting exceptional seedling robustness, despite a slightly longer germination time (4.57 days). Treatments like mechanical scarification, hydrogen peroxide, and dry heat exposure proved ineffective or even detrimental, failing to break dormancy or resulting in embryo damage. Overall, the findings highlight that ultrasonication is a superior pretreatment strategy. Ultrasonication offers a reliable, non-chemical, and scalable solution to overcome seed dormancy and enhance early growth performance in *C. sativa* cultivation.

Statistical analysis revealed significant differences in germination performance among the various pretreatment methods applied to seeds. A chi-square test of independence indicated a strong association between pretreatment type and germination success ($\chi^2(12, N = 195) = 58.22$, $p < 0.0001$), confirming that seed pretreatment plays a critical role in overcoming dormancy and enhancing germination. Pairwise two-proportion z-tests, adjusted using the Holm–Bonferroni method, showed that four pretreatments produced significantly higher germination percentages compared to the control group (normal water soaking, 26.66%).

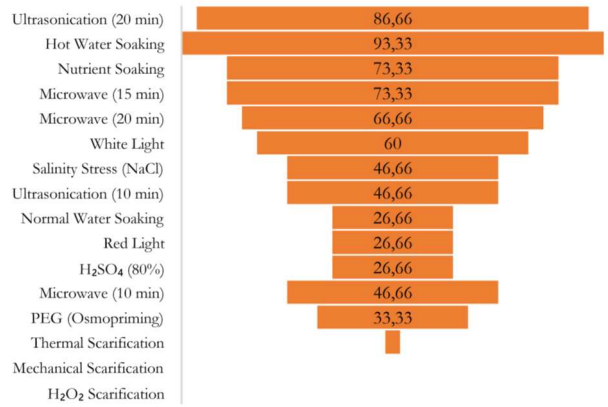

Figure 14. Effect of different seed pre-treatment methods on germination percentage. Hot water soaking (93.33%) and ultrasonication for 20 minutes (86.66%) showed the highest germination, while mechanical and H_2O scarification resulted in no germination.
Figura 14. Efeito de diferentes métodos de pré-tratamento de sementes sobre a porcentagem de germinação. A imersão em água quente (93,33%) e a ultrassonicção por 20 minutos (86,66%)

apresentaram as maiores taxas de germinação, enquanto a escarificação mecânica e a escarificação com H_2O não resultaram em germinação.

These results statistically validate the effectiveness of select physical and advanced pretreatment strategies, particularly hot water soaking and ultrasonication, for improving germination efficiency in *C. sativa*.

3.6. Phytochemical Analysis

3.6.1. Extraction of bioactive compounds

The extraction of bioactive compounds from sprouts subjected to various pretreatment methods revealed significant differences in phytochemical yield and antioxidant activity. Among the tested approaches, microwave pretreatment proved the most effective. Autoclave pretreatment yielded improvements over the control as well, but was less effective than ultrasound or microwave. These findings suggest that advanced pretreatment methods, especially microwave irradiation, significantly improve the extraction efficiency of bioactive compounds by disrupting cellular structures and enhancing solvent penetration during extraction.

3.6.2. Total Phenolic Content (TPC)

The total phenolic content of sprouts varied significantly across different pretreatment methods. The microwave-treated samples exhibited the highest TPC, reaching 9.22 ± 0.34 mg GAE/g, which was substantially greater than the untreated control (5.34 ± 0.26 mg GAE/g). Ultrasound pretreatment also improved TPC levels, yielding 8.31 ± 0.41 mg GAE/g, while autoclave pretreatment resulted in a moderate increase to 6.91 ± 0.25 mg GAE/g. These results suggest that thermal and non-thermal pretreatments enhance phenolic compound extraction, with microwave being the most effective due to its ability to disrupt cellular matrices and improve solvent diffusion.

3.6.3. Radical scavenging activity

The antioxidant activity of the sprout extracts, assessed via the DPPH assay, mirrored the TPC trend. Microwave-pretreated samples demonstrated the highest radical scavenging activity at 76.22 ± 1.44%, followed by ultrasound (70.14 ± 1.38%) and autoclave pretreatment (61.91 ± 1.28%). In contrast, the control sample showed the lowest antioxidant activity, with 52.31 ± 1.22% inhibition. The significant enhancement in antioxidant activity observed in microwave-treated samples may be attributed to the elevated presence of phenolic compounds, which are known for their free-radical neutralizing capabilities. This reinforces the strong correlation between TPC levels and antioxidant capacity.

Similarly, the antioxidant activity, measured through the DPPH assay, was highest in microwave-treated samples (76.22 ± 1.44%), which correlates with their elevated TPC. This suggests a strong association between phenolic content and radical scavenging ability, as phenolics are well-known antioxidants that neutralize free radicals via hydrogen donation. While ultrasound pretreatment also enhanced both TPC (8.31 ± 0.41 mg GAE/g) and DPPH scavenging activity (70.14 ± 1.38%), its effects were slightly lower than those of microwaves. This is likely due to the cavitation phenomenon induced by ultrasound, which disrupts cell structures and enhances mass transfer, albeit less aggressively than microwaves. Autoclave pretreatment resulted in moderate improvements in both parameters (TPC: 6.91 ± 0.25 mg GAE/g; DPPH: 61.91 ± 1.28%), suggesting that prolonged thermal exposure may cause partial degradation of heat-sensitive phenolics, offsetting the benefits of cell rupture. The control group, which received no pretreatment, showed the lowest TPC and antioxidant activity, confirming that pretreatment is essential to optimize extraction efficiency. These findings highlight the pivotal role of pretreatment technologies in enhancing the recovery of valuable phytochemicals and antioxidant potential from plant-based materials.

4. DISCUSSION

Surface sterilization is not an easy task because microbes on the surface of the seed should be removed, but the embryo must not be damaged by the procedure (SAUER et al., 1986). Light can enhance the content and activity of certain enzymes in seeds, thereby promoting seed germination. The seed's response to light is a mechanism that ensures germination occurs under conditions favorable for seedling growth (SHAHVERDI et al., 2019). Light triggers the activation of specific enzymes that are involved in the breakdown of stored reserves, providing the energy needed for the growing seedling (WANG et al., 2014). Recently, it has been reported that a hydrogen peroxide (H_2O_2) solution can significantly enhance the germination rate of *C. sativa* seeds (SOROKIN et al., 2021). H_2O_2 is not only a well-established antimicrobial agent (Weston, 2000) but is also a signaling molecule for many physiological, developmental, and stress-tolerance processes of plants (WOJTYLA et al., 2016). H_2O_2 has strong oxidizing capacities (KU et al., 2021). It can interact with most biomolecules, including nucleic acids, proteins, and lipids. Recent evidence has shown that the selective oxidation of proteins and mRNAs can act as a positive regulator of seed germination (JOB et al., 2005).

Scarification is a commonly used seed pretreatment method aimed at breaking dormancy and promoting uniform germination. This technique involves physically altering the seed coat using tools such as sandpaper, knives, and needles to make it permeable. Methods include puncturing, burning, scratching, filing, or shattering the seed coat to facilitate water and air uptake. When applied appropriately, scarification enhances the seed's ability to imbibe water, allowing oxygen and moisture to penetrate more efficiently and initiate germination (PLANT, S.G.O.A.). The effectiveness of thermal scarification varies considerably depending on heating devices, treatment time, and temperature. Thermal scarification had a little influence on hard seed reduction when treatment temperatures were between 40-50°C (HARRINGTON, 1916). The agricultural value of impermeable seeds is problematic. Neither reduction of hard seed nor improvement of germination was observed in the seed of alfalfa (*M. sativa* L) when they were heated at 40 °C (RUTAR et al., 2001). Hot water soaking is highly effective in breaking seed coat-imposed dormancy. This method significantly enhanced both the speed and percentage of germination, making it a practical and efficient technique for improving propagation success (KOOBONYE et al., 2018). Hot water treatments have been used successfully on a large number of tropical and subtropical seeds (DOUSSI; THANOS, 1994). Empirical evidence supports the effectiveness of this method. For instance, Koobonye et al. (2018) demonstrated that hot water pretreatment

significantly improved germination in *Vigna unguiculata* (cowpea), reducing mean germination time and increasing germination percentage when compared to untreated seeds.

White light, which includes the full spectrum of visible wavelengths (400–700 nm), can influence seed germination by activating phytochrome photoreceptors, particularly phytochrome B. The presence of white light, therefore, can shift the hormonal balance in favor of germination under suitable conditions (SEO et al., 2009). However, the efficacy of red light in stimulating germination is species-specific and depends greatly on the seed's innate dormancy mechanisms. In some species, red light alone is sufficient to trigger germination. For example, *Arabidopsis thaliana* and *Lactuca sativa* respond strongly to red light, showing significantly improved germination compared to darkness (SHINOMURA et al., 1994). On the other hand, seeds that exhibit non-photoblastic dormancy, like *C. sativa*, do not rely solely on light cues for germination initiation and instead require physical or physiological pretreatments such as scarification or thermal treatment to become responsive to hormonal or environmental signals (Bewley et al., 2012; Schluttenhofer & Yuan, 2017). In many cases, red light must be followed by far-red light or be combined with other environmental cues such as temperature, moisture, or hormonal changes to complete the light-dependent germination response (Casal et al., 1998).

Acid scarification is considered the most effective scarification method used for seed scarification. Sulfuric acid is the most popular and effective chemical to reduce the hard seed coat of legumes (KIMURA et al., 2012). Aliero (2004) demonstrated that treating *Parkia biglobosa* seeds with concentrated H_2SO_4 for 10-15 minutes significantly enhanced germination (up to 90%), compared to only 20-30% in untreated seeds. Studies on *Lupinus* species showed that some of the other species, genotypes, and cultivars have similar seed coat dormancy, and sulfuric acid treatment is required to get rapid and uniform germination (KAK et al., 2009). KNO_3 is one of the most widely used priming agents due to its dual role in breaking seed dormancy and supplying nitrate, a crucial signaling molecule. Studies by Afzal et al. (2006) demonstrated that KNO_3 priming improved germination and seedling performance in wheat. In these cases, seeds primed with 1-2% KNO_3 showed faster and more uniform emergence, higher seedling length, and dry biomass. Also, $CaCl_2$ has been shown to improve seedling vigor and enhance germination under both optimal and stress-prone conditions. For instance, Hussain et al. (2006) reported that maize seeds primed with 1.5% $CaCl_2$ had improved emergence and seedling vigor even under suboptimal field conditions. Saline stress is primarily caused by high concentrations of sodium chloride or other salts, which reduce osmotic potential and limit water uptake by seeds during imbibition (MUNNS; TESTER, 2008). Under such stress conditions, many seeds fail to initiate or complete germination. This failure to complete germination reinforces dormancy and is the result of impaired water uptake, inhibited metabolic enzyme function, and altered hormonal balance, particularly a reduction in gibberellic acid synthesis and increased abscisic acid activity (JAMIL et al., 2007). Jamil; Rha (2004) reported that salinity levels of 100-150 mM NaCl significantly reduced germination percentages in rice and sugar beet. The use of PEG, a high-molecular-weight osmoticum, is a well-established method to simulate drought-like or water-deficit conditions by creating controlled osmotic stress without ion toxicity (HARDEGREE; EMMERICH, 1990). The low germination percentage observed under PEG treatment in this study is consistent with previous reports in other species. For instance, Michel; Kaufmann (1973) reported that increasing PEG concentration led to a sharp decline in germination of corn, wheat, and soybean. Sadeghian; Yavari (2004), similarly observed that sugar beet seeds exhibited reduced germination and shoot growth under -0.6 to -1.2 MPa PEG-induced osmotic stress.

Microwave pretreatment is a non-chemical method that uses electromagnetic radiation to break seed dormancy and enhance physiological activity. This technique can alter seed coat permeability and stimulate enzyme activity, potentially improving seed germination and vigor. This study investigates the effect of different durations of microwave exposure on the germination and early growth parameters of seeds. Several studies have investigated the effects of microwave treatment on seed germination in various plant species, including green gram (*Vigna radiata*), wheat (*Triticum aestivum*), moth bean (*Vigna aconitifolia*), and Bengal gram (*Cicer arietinum*) (MOHSENKHAH et al., 2018). Ultrasounds are acoustic waves at frequencies higher than 20 kHz. Ultrasounds are often used in the agro-industry to enhance processes such as drying, extraction, emulsification, and defoaming (MASON et al., 2005). Ultrasound treatments have been reported to stimulate germination in different types of plants, such as wheat, watermelon, and pepper (Shin et al., 2011), barley (Yaldagard et al., 2008), Calanthe hybrids, and bean (RUBTSOVA, 1967). The effects of ultrasonics on seed germination and productivity have been reported in fodder beans (RUBTSOVA, 1967), alfalfa and broccoli (KIM et al., 2006), fern spores (SOSSOUNTZOV, 1954), and sorghum (PATERNO et al., 2015). The current study demonstrates the significant influence of various pretreatment methods on the total phenolic content (TPC) and antioxidant activity (as measured by the DPPH radical scavenging assay) of sprouts. Among the treatments, microwave pretreatment consistently produced the highest levels of phenolic compounds and antioxidant activity, indicating its superior efficiency in enhancing the release of bioactive compounds. The increase in TPC observed in microwave-pretreated samples (9.22 ± 0.34 mg GAE/g) may be attributed to the thermal and non-thermal effects of microwave energy, which rapidly heats intracellular moisture, generating pressure that ruptures cell walls and facilitates solvent access to phenolic compounds. This mechanism aligns with findings from earlier studies that report enhanced polyphenol extraction under microwave-assisted extraction (MAE) due to cell wall disruption and improved mass transfer (CHEMAT et al., 2017).

5. CONCLUSIONS

This study systematically explored the influence of various physical, chemical, and advanced pretreatment methods on the germination efficiency and seedling vigor of *C. sativa*. Reulst obtained evidence that seed pretreatments significantly improve germination outcomes, with notable differences in effectiveness among the tested methods. Ultrasonication for 20 minutes proved to be the most effective overall, achieving a high germination percentage of 86.66%, thereby reducing mean germination time to 2.84

days, and exhibiting a strong seedling vigor index of 852.46, likely due to the enhanced water absorption and metabolic activation resulting from acoustic cavitation. Hot water soaking, though simpler, yielded the highest germination rate of 93.33% and presents a practical, low-cost method suitable for broader application. Nutrient soaking using potassium nitrate and calcium chloride produced the highest seedling vigor (SVI: 924.89), suggesting improved physiological performance and robust early growth. Advanced techniques such as microwave treatment also demonstrated significant improvements, particularly in enhancing phytochemical extraction, including increased total phenolic content and antioxidant activity. In contrast, traditional approaches like mechanical scarification, hydrogen peroxide treatment, and thermal exposure were largely ineffective or detrimental. Collectively, these findings emphasize the importance of selecting appropriate pretreatment strategies tailored to the biological and physiological characteristics of *cannabis sativa* seeds. Advanced, non-chemical methods such as ultrasonication and microwave radiation offer promising, scalable solutions for overcoming seed dormancy and enhancing both propagation success and phytochemical yield, with meaningful implications for research, agriculture, and pharmaceutical applications.

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Data availability: Study data can be obtained by email from the corresponding author. It is not available on the website as the research project is still under development.

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