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Optimizing Lettuce Growth Protocols in Controlled Environments via Light Quality Manipulation, Hormonal, and Nano-Interventions.

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I. Abstract

This laboratory study conducted at the College of Science University of Wasit investigated the effects of light wavelengths nanomaterials and hormonal treatments on the germination of lettuce seeds where the results revealed a strong photoblastic response with a maximum germination rate of 85.00 percent under red light compared to 50.33 percent in darkness and a minimum of 13.33 percent under far-red light and the inhibitory effect of far-red light was significantly reversed by red light as a final 10-minute exposure to red light stimulated germination to 73.67 percent while for chemical and nano stimulation the highest germination rates were recorded at 80.33 percent using Gibberellic acid at 150 ppm and 75.00 percent using nanomaterials at a concentration of 0.05 which leads to the conclusion that lettuce seed germination is highly dependent on the light spectrum and chronological sequence of exposure while hormonal and nano-interventions effectively enhance the overall germination potential.

Keywords : *Lettuce (Lactuca sativa), red and far-red light effect, Nano-iron oxide, Nano-magnesium oxide, Gibberellic acid , kinetin.*

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II. Introduction

Lettuce is a crop of Asian origin and is widely produced in the United States, Europe, Mexico, Chile, Argentina, Brazil, Peru, China, Japan, and Australia (Patil et al.,2017). In addition, lettuce production has increased markedly in South Asian countries in recent years. Global production of lettuce and chicory is estimated at approximately 17,228 metric tons, with China leading global lettuce production at 53%, followed by the United States at 17% (Taghizadeh et al.,2021). Studies conducted by Kim et al. (2016) and Medina-Lozano et al. (2021) has demonstrated the high nutritional value of lettuce. The leafy tissues are rich in dietary fiber, antioxidants, and compounds with antiproliferative activity against abnormal cell growth (such as vitamins C and E, polyphenols, and carotenoids), in addition to other metabolic products, including anthocyanins, chlorophylls, glycosylated flavonoids, and sesquiterpene lactones. Lettuce leaves also contain folic acid, essential minerals such as calcium, iron, magnesium, potassium, and sodium as well as vitamin A (Yang et al., 2022; Medina-Lozano et al., 2024).

The light of the rapid advancement in modern agricultural technologies, particularly protected cultivation and controlled-environment agriculture, increasing attention has been directed toward understanding the complex interactions between physical and chemical factors that regulate plant growth and productivity. Among the most influential of these factors are light spectral quality, plant hormones, and nanotechnology-based compounds, which collectively play critical roles in modulating plant physiological and developmental processes (Schwab et al., 2016). Most phytochemical levels in indoor-grown lettuce were not affected by the addition of far-red light (17% of total photon flux density) to white LEDs. Nonetheless, phytochemical concentrations decreased when far-red light was combined with UVA, indicating that the interaction between far-red light and UVA influences the accumulation of these compounds (He et al. 2021)

Moreover, light wavelengths, particularly red and far-red radiation, function not only as an energy source for photosynthesis but also as critical environmental signals that regulate plant growth and development through intricate hormonal and molecular networks mediated by phytochrome photoreceptors (Liu and Van Lersel., 2021). Consequently, investigating the integrated effects of nanoparticle, plant hormones, and specific light wavelengths on lettuce, represents a scientifically significant approach to advancing our understanding of plant growth regulation and improving crop productivity and quality in modern agricultural systems. Such knowledge is essential for the development of more efficient and sustainable production strategies capable of addressing current challenges related to food security and climate change (Rouphael and Colla, 2020).

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III.Materials and Methods

1. Experimental Site

A laboratory experiment was conducted at the College of Science, Department of Biology during the 2025. The experiment investigated the effects of different light wavelengths on germination percentages, as well as the effects of nanoparticles and plant growth regulators on Lettuce (*Lactuca sativa*). All experiments were carried out in a controlled growth chamber within the Plant Graduate Studies Laboratory.

2.Seeds

The seeds of Lettuce (*Lactuca sativa*).was obtained from certified agricultural supply stores in Wasit Governorate. seeds were commercially packaged and of Indian origin. Prior to the experiment, seed viability was assessed through standard germination tests to ensure uniformity and physiological quality of the seed lots. Only seed batches exhibiting high germination percentages and normal seedling development were selected for subsequent experimental treatments.

3. Seed Germination and Sterilization

The seeds were sterilized using a seed fumigant material. The substance was diluted with distilled water, mixed with the seeds individually with continuous stirring, and distributed over the surface of the seeds to ensure adequate sterilization. Afterwards, the seeds were spread on a piece of sterile gauze to dry. Petri dishes were prepared, with 20 seeds placed in each dish. The dishes were placed inside a light-tight, sealed box. The box was tightly closed to prevent any light penetration. Additionally, three other replicates for each plant species were placed directly under light to test whether the seeds germinated in darkness or required light. Both the sealed box and the exposed dishes were then placed inside a growth incubator (model: Environmental System
LabTech Daihan Labtech). The germination percentage was calculated. Dark germination was predominant for broccoli (Ibrahim and Sharhan,2025).

Figure1: Seed dusting material

Figure2: Dusted broccoli seeds

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4. Light Conditions

The experiments were conducted using specific light wavelengths. The wooden boxes containing the experimental units were placed inside a plant growth chamber equipped with built-in LED lighting systems. An optical filter was installed to ensure that only the required filtered light reached the samples, following the methodology described by Al-Ukaila (1990).

For studies on the effects of specific wavelengths:

- Red light (600–700 nm) was obtained using a red optical filter with an irradiance of 0.11 cal /cm²/min.
- Far-red light (700–760 nm) was obtained using a filter composed of two layers (yellow and purple) with an irradiance of 0.08 cal /cm²/min.
- Blue light (400–500 nm) was obtained using a dedicated blue optical filter.
- White light (400–700 nm) was using the same LED lamps after removing the optical filters, allowing unfiltered white light to pass through the same aperture.

The optical filters were placed in their designated positions at the top of each box according to the requirements of each experiment, following the preparation and arrangement of the Petri dishes containing the seeds.

5. Ultraviolet (UV-C) Light Treatment

The ultraviolet light experiment was conducted using a UV candle of type C (UV-C) as the radiation source. A specially designed ceramic box was constructed and placed inside the laboratory to ensure isolation from external light interference and to maintain stable experimental conditions. Petri dishes containing the seeds were placed inside the box, with the UV-C candle positioned at a fixed distance of 30 cm above the dishes. The seeds were continuously exposed to UV-C radiation for a period of 7 days under a 24-hour exposure regime.

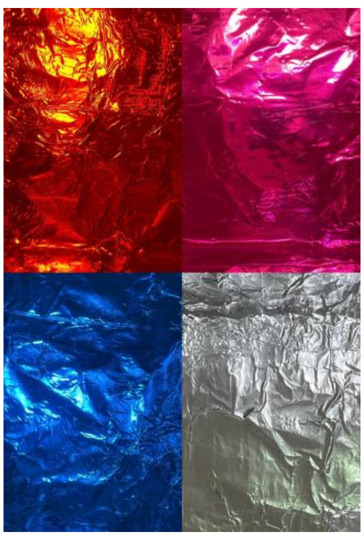

Figure3:Optical Filter Experimental

Figure4:Seeds under (UV) radiation

6. Preparation of Hormone Solutions

The solutions of gibberellin (GA3) and kinetin were prepared following a standard methodology emphasizing accuracy and consistency. The process began by weighing 0.100 grams of each hormone separately using an analytical balance. Each was then dissolved in 1 ml of absolute ethanol to ensure complete dissolution before being transferred to a 100 ml volumetric flask and brought to volume with sterilized distilled water, yielding a 1000 ppm stock solution for each hormone. Subsequently, precise serial dilutions were performed from this stock solution using sterilized distilled water to obtain the required working concentrations for the experiment: 10, 50, 100, and 150 ppm, applying exact volumetric calculations. To preserve the hormones' efficacy, all solutions were stored in amber containers at a temperature of 4°C.(Sharhan et al.,2022)

7. Preparation of nanoparticles

The two types of nanoparticles, namely iron oxide (Fe_2O_3) and magnesium oxide (MgO), were prepared as aqueous suspensions for experimental application. The preparation process began by dissolving 0.1 g of each nanoparticle powder in 5 mL of ethanol to ensure initial de-agglomeration and stabilization. This mixture was then quantitatively transferred to a volumetric flask, and the volume was brought to 500 mL using deionized water under continuous stirring in a water bath to ensure uniform dispersion, resulting in a stock solution.

From this stock solution, the required working concentrations (0.01, 0.03, 0.05, and 0.07 $mg \cdot mL^{-1}$) were prepared through further dilution. The nanoparticle compounds were obtained from Dr. Ahmed Thamir, Department of Physics, College of Science. All suspensions were freshly prepared prior to use, with 10 mL placed in each Petri dish for experimental application (Juma'a, 2025).

8. Measurement of Germination Percentage of the Tested Plants

The seeds were distributed in Petri dishes with a diameter of 9 cm, placing 20 seeds per dish for each plant species separately. Two sheets of filter paper were placed inside each dish to maintain adequate moisture. After seven days of incubation, in accordance with the rules of the International Seed Testing Association (ISTA, 2024), the germination percentage was calculated.

The germination percentage was determined using the following formula:

Germination percentage (%) = (Number of germinated seeds / Number of sown seeds) × 100%

9. Statistical Analysis:

Examining the differences between statistically significant treatments was made easier using the Complete Block Design (CBD) analysis of variance test. The mean comparison was evaluated using the least significant differences (LSD) at 0.05 percent levels in program (SPSS).

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Results

Laboratory results indicate a fundamental difference in germination efficiency, with seeds in the light achieving a high germination rate of 85%, while this rate dropped to 50.33% under dark Physiologically, this variation is attributed to the mechanism of action of phytochromes

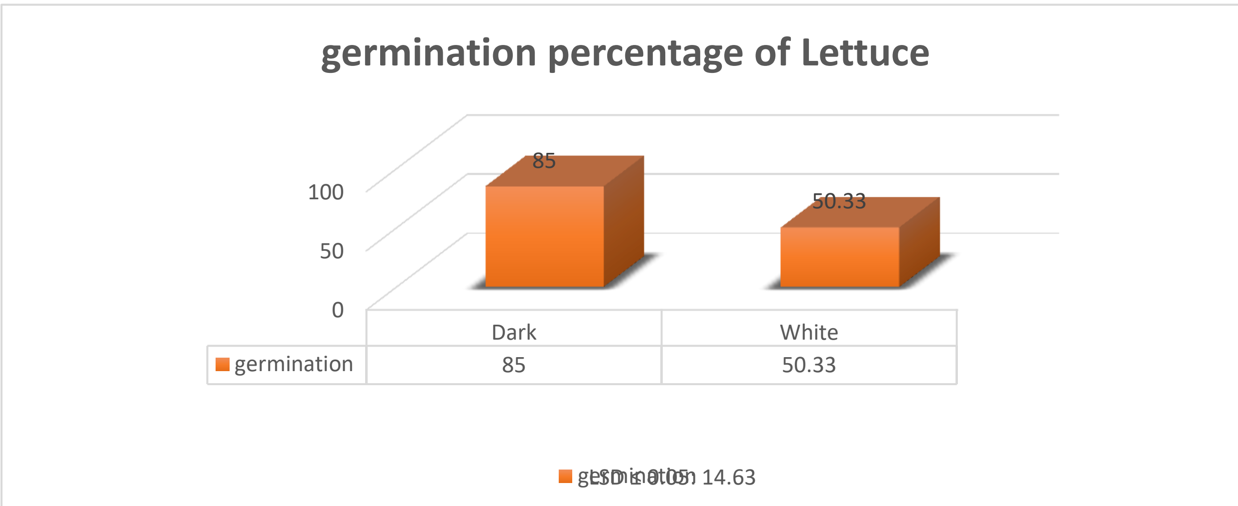

Figure 5. The germination percentage of Lettuce (Lactuca sativa)

Physiologically, this variation is attributed to the mechanism of action of phytochromes. The wavelengths in white light stimulate 'photodormancy' in Lettuce seeds, leading to the inhibition of metabolic enzymes required for breaking down stored reserves.From a hormonal perspective, darkness increases tissue sensitivity to gibberellins, the primary hormone responsible for breaking seed dormancy and activating alpha-amylase, which breaks down starch into simple sugars that provide the embryo with the energy needed for emergence. In contrast, continuous exposure to white light may increase abscisic acid (ABA) concentration, a germination-inhibiting hormone, which explains the drop in germination rate to less than half upon light exposure. This physiological behavior classifies Lettuce seeds (in this experiment) as negatively photoblastic, meaning they prefer the absence of light to achieve maximum germination potential.

The **figures 6** below present the effect of different light wavelengths on the germination of of Lettuce *(Lactuca sativa*) demonstrating distinct inhibitory responses, The results show a sharp contrast in Lettuce seed germination rates in response to the quality of incident light, and this response can be analysed from a molecular physiological perspective as follows. Red light (83.33% germination) recorded the highest rate among the wavelengths, physiologically because it activates phytochrome by converting it from the inactive form (Pr) to the active form

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(Pfr). This active form acts as a biological switch that promotes the gene expression responsible for gibberellin (GA) synthesis and inhibits abscisic acid (ABA), thereby breaking seed dormancy and initiating metabolic processes. In contrast, far-red light (13.33% germination) resulted in the lowest rate, which is physiologically logical since far-red light converts phytochrome back to its inactive Pr form, disrupting germination signals and mimicking conditions of deep dormancy or dense canopy cover (shade avoidance), explaining the sharp drop compared to red light. Intermediate responses were replaced differently with blue light and UV (71.67% and 53.33%, respectively), where blue light is perceived by cryptochromes. Although blue light can stimulate germination in some species, in Lettuce it leads to a less efficient response than red light, because blue light sometimes interferes with hormonal pathways that limit the speed of initial cell division relative to the red light stimulus. Scientifically, based on these results, Lettuce seeds are classified as highly photoblastic, with the phytochrome system playing a pivotal role in determining germination outcome, where red light acts as the strongest biological promoter while Far-red light serves as the primary physiological inhibitor of this process.

Figure 6. The effect of wavelengths on the germination percentage of Lettuce seeds.

The data presented in the **tables1** illustrate the effect of MgO and Fe_2O_3 nanoparticles on the germination of Lettuce seeds, where the results demonstrate a significant and clear stimulatory effect for both types of nanoparticles compared to the control treatment which stood at 13.33. At a concentration of 0.01 mg/mL, Magnesium Oxide (MgO) nanoparticles achieved a stimulation percentage of 325.13% while Iron Oxide (Fe_2O_3) recorded 262.57%, indicating a strong initial response to magnesium at low concentrations. As the concentration increased to 0.03 mg/mL, growth rates continued to rise bringing the stimulation percentage for MgO to 387.62% and for FeO to 400.15%, which reflects the efficiency of nanoparticles in activating the biological and chemical processes of the plant. The experiment recorded the peak stimulatory activity at a concentration of 0.05 mg/mL for both materials, where MgO reached its highest level at 437.66% and FeO achieved the absolute peak in the table with a stimulation percentage of 462.64%. However, upon moving to the high concentration of 0.07 mg/mL, a decline in stimulation rates was observed, dropping to 322.58% for magnesium and 300.08% for iron, suggesting both statistically and scientifically that the saturation point was reached or that inhibitory effects began to emerge due to the concentration exceeding the optimal limit. Considering the Least Significant Difference (LSD 0.05) values, it is evident that the jump from the control treatment to the first nano-concentration represents the most fundamental difference in the experiment, while the differences between the intermediate concentrations were statistically less pronounced despite the numerical superiority of the 0.05 mg/mL concentration as the best stimulatory treatment for lettuce

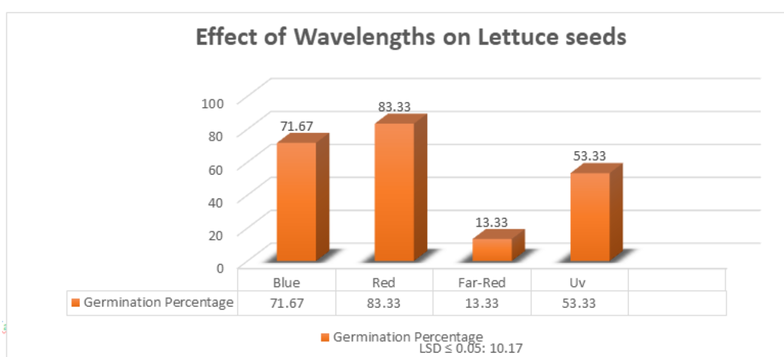

Table 1 : The effect of nanoparticles on the germination percentage of Lettuce (Lactuca sativa) seeds

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The data presented in the **tables 2** demonstrate a clear and significant superiority of the plant growth regulators gibberellin (GA_3) and kinetin (KA) in stimulating the germination of Lettuce seeds compared to the control treatment which stood at 13.33. Gibberellin treatment (GA_3) showed a consistent upward trend in stimulation, starting at 300.08% for the 10 mg/L concentration and increasing steadily until it reached its maximum stimulation percentage of 502.63% at the highest concentration of 150 mg/L, a strong promotive effect attributed to its fundamental role in activating hydrolytic enzymes and counteracting the inhibitory effect of far-red light by acting as a biological substitute for the active phytochrome (Pfr) form. On the other hand, Kinetin (KA) displayed a high stimulatory capacity that peaked at 452.66% at a concentration of 100 mg/L, but its effectiveness declined to 352.59% when the concentration was raised to 150 mg/L, a response that can be physiologically explained by kinetin's role in promoting cell division while the decline at the highest dose indicates that the seeds reached a saturation state or that a hormonal imbalance occurred. Statistical analysis using the LSD (0.05) values confirms that the differences between all hormone concentrations and the control treatment (13.33) were highly significant, underscoring the pronounced sensitivity of lettuce seeds to these growth regulators. It can be concluded that while both regulators are highly effective, GA_3 proved to be the more stable and efficient stimulant for lettuce seed germination under these conditions, as it maintained a continuous increase in germination rates across all tested concentrations, whereas kinetin exhibited its optimal response at the 100 mg/L concentration before declining

Table 2 : The effect of plant hormones(ppm) on the germination percentage of Lettuce (Lactuca sativa) seeds

Treatments	Gibberellin (GA3)	kinetin
6 Day Far-red Control	13.33	13.33
10	53.33	41.67
50	66.67	58.33
100	75.00	73.67
150	80.33	60.33
LSD (0.05)	11.25	13.68

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Treatments	Nanoparticles MgO	Nanoparticles Fe_2O_3
6 Day Far-red Control	13.33	13.33
0.01	56.67	48.33
0.03	65.33	66.67
0.05	71.67	75.00
0.07	56.67	53.33
LSD (0.05)	15.91	11.006

Table 3 : The effect of far-red light on c at different exposure periods percentage of Lettuce *(Lactuca sativa*) seeds

In the table below, Table A illustrates the interaction of factors affecting the lettuce plant compared to the two control treatments where the first is six days of darkness 6D with a value of 85.00 and the second is six days of far-red light 6 FR with a value of 13.33 as the analysis shows that the combined effect of these factors depends strongly on the chronological order of exposure because when mixing 1 FR with different days of D the mean values ranged between 50.33 and 60.00 which are all significantly lower than the darkness control 85.00 and significantly higher than the far-red control 13.33 based on the LSD 10.36 and it is clear that the distribution of D before and after the single FR makes a difference where the best result among mixed treatments occurred when FR was placed at the end of the period 5D_1FR with a mean of 60.00 followed by 1D_1FR_4D at 56.67 while the lowest value was recorded in the 3D_1FR_2D treatment at 50.33 which indicates that prior exposure to D for several days before FR mitigates the negative effect of far-red light whereas earlier exposure to FR limits the plant's ability to benefit from subsequent D periods and statistically there are no significant differences among the mixed treatments 60.00 56.67 55.00 53.33 and 50.33 as the variations between them are less than the LSD value of 10.36 while the 6D control remains significantly superior to all treatments and the 6 FR control is significantly lower than all others.

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Treatments	germination percentage
6D	**85.00**
6 FR	**13.33**
5D_1FR	**60.00**
4D_1FR_1D	**53.33**
3D_1FR_ 2D	**50.33**
2 D _1FR _3 D	**55.00**
1 D _1FR_4 D	**56.67**
LSD (0.05)	**10.36**

Figure 7. The effect of far-red light on germination at different exposure periods of Lettuce *(Lactuca sativa*) seeds

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Figure 7 illustrates how seed germination percentage is influenced by the duration of initial exposure to inhibitory light, revealing an inverse relationship between the length of far-red (FR) light treatment and the final germination percentage. When seeds were exposed to FR light for only two days at the start of the experiment (2FR_4D), 56.67% of the seeds successfully overcame the inhibitory effect and germinated upon transfer to light. However, increasing the initial FR exposure to four days (4FR_2D) caused a significant reduction in germination to 38.33%. This decline, supported by the LSD value of 11.53, confirms that prolonging the time phytochrome remains in its inactive form (Pr) during the early hours of germination leads a larger proportion of seeds into a state of secondary dormancy that is difficult to break even after transfer to inductive light conditions .

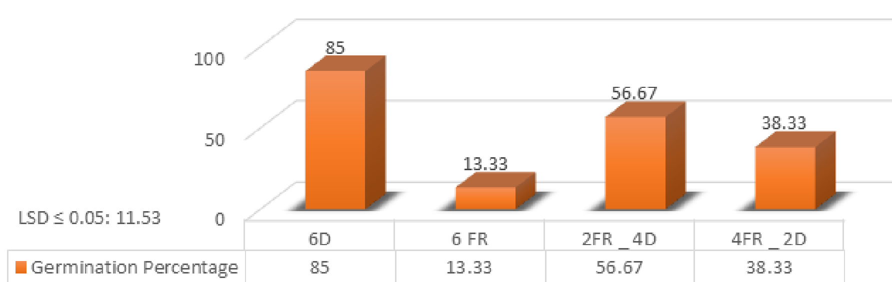

Table 4 provides practical evidence of the phytochrome light-switch mechanism in controlling Lettuce seed

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Treatments	germination percentage
6D	85.00
6 FR	13.33
4 D -1FR-D	53.33
4 D- 1FR -_5 min Red -D	61.67
4D-1FR -10 min Red -D	75.00
4D-1FR -_10 min Red – 10 min FR-D	58.33
4D -1FR_10min Red-10min FR-10min Red-D	73.67
LSD (0.05)	11.78

Table 4. The effect of the interaction between far-red and red light on the germination percentage of Lettuce (Lactuca sativa) seeds

germination, clearly highlighting the phenomenon of photo reversibility through the ability of brief red light flashes to cancel the inhibitory effect of FR light. The treatments reveal that the final germination percentage follows the type of "last flash" received by the seeds. When FR was the concluding flash, germination dropped to 58.33%, whereas a final flash of red light lasting only 10 minutes dramatically increased germination to 73.67% — a value very close to that of complete light germination (85.00%). This is scientifically explained by the fact that red light converts the photoreceptor pigment to its active form (Pfr), which stimulates the production of germination hormones such as gibberellins, thereby nullifying the inhibition previously caused by FR. The LSD value of 11.78 confirms the efficacy of these short light flashes in inducing substantial changes in germination percentages

*4D: four day, 1FR: one day far red ,10,5 min : minute

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IV. Discussion

The differences in Lettuce seed germination rates under different wavelengths are attributed to the sensitivity of the phytochrome system (particularly PhyA and PhyB), where red light (660 nm) converts phytochrome to its active form (Pfr), which translocates to the nucleus and binds to PIF transcription factors, relieving gene repression and activating genes for dormancy-breaking enzymes such as expansins and proteases, while far-red light (730 nm) reverses this process, converting Pfr back to the inactive Pr form, leading to a sharp drop in germination due to inhibition of primary metabolic processes that precede radicle emergence(Liu et al., 2022; Santini et al., 2023).

In terms of plant growth regulators, the stimulating effect of hormones added to photo inhibited samples is due to gibberellins (GA_3) acting as a biological switch that bypasses the need for photo stimulation (Oka et al., 2025). The results showed that treating Lettuce seeds with gibberellic acid (GA_3) and kinetin under far-red light enhances germination by mimicking the role of red light in activating phytochrome B. This finding is consistent with El-Rasafi (2025), who reported that GA stimulates the germination of dark-requiring seeds, such as Phacelia and Nemophila, even under far-red light conditions. In contrast, prolonged exposure to far-red light suppresses germination by inactivating phytochrome receptors and disrupting hormonal balance—through reducing GA levels and increasing abscisic acid (ABA) as noted by Al-Ukaili (1990)

Nanoparticles improve germination through multiple mechanisms, they temporarily increase seed coat permeability to water and oxygen, induce mild oxidative stress (a transient rise in ROS) that acts as a hormetic signal to activate antioxidant enzymes (SOD, CAT, APX) and growth-related pathways, and act as catalytic agents that accelerate enzymatic reactions supporting radicle emergence, including upregulation of endogenous auxin (IAA) which helps the radicle penetrate covering layers under suboptimal conditions(Tawfik et al., 2021; Koelmel et al., 2013; Yousuf et al., 2025) . Photo reversibility experiments involving dark periods and far-red/red light show that the final germination percentage depends on the last wavelength received or the subsequent dark interval, because the Pfr/Ptot ratio determines the response; far-red lowers this ratio while red raises it, and during a sufficiently long dark period, active Pfr slowly reverts to Pr via dark reversion or de novo synthesis, eventually reaching a Pfr/Ptot equilibrium that allows growth signals (GA synthesis and ABA inhibition) to override the inhibition imposed by far-red light – this explains the restoration of high germination rates when adequate dark periods are introduced or when far-red is followed by red light (Al-Jaafari et al., 2024; Rizza et al., 2019; Azizi et al., 2025). Finally, The response of Lettuce indicates that growth in darkness follows etiolation, where elongation of the plumule and radicle is promoted by elevated auxin activity and increased cell wall extensibility, representing an adaptive strategy to search for light (Taiz et al., 2015 ;Sharhan et al., 2022) .

In contrast, exposure to light shifts the plant toward photomorphogenesis, in which photoreceptors such as phytochromes and cryptochromes are activated, leading to suppression of longitudinal elongation and enhancement of structural differentiation and tissue development (Kami et al., 2010). Red light shows a relatively stimulatory effect on growth through phytochrome regulation, whereas blue and far-red light tend to inhibit elongation more strongly due to their influence on hormonal balance and light signaling pathways. (Ali et al., 2020; Feizi et al., 2013)

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V. Conclusions

Red and far-red light represent key environmental signals that regulate seed germination through activation of the phytochrome system, which in turn modulates hormonal pathways and enhances the physiological activity of seeds. Accumulating evidence indicates that targeted manipulation of these wavelengths can improve germination efficiency and seedling vigor across a wide range of plant species. Accordingly, the strategic use of red and far-red light represents a promising approach for advancing agricultural practices and developing optimized germination technologies aimed at increasing crop productivity.

In addition, the results demonstrated that the application of nanoparticle and plant growth regulators significantly stimulated seed germination and promoted plant growth, highlighting their potential as supportive tools to enhance physiological performance and improve overall plant productivity.

Novelty Statement

This study provides new insights into how light wavelengths, plant hormones, and nanoparticle collectively influence the germination physiology of Lettuce (*Lactuca sativa)* seeds

By integrating hormonal and nanotechnological treatments with photo biological responses, the research identifies previously unreported interactions that clarify the dark-requiring germination behavior of Lettuce seeds and determine optimal stimulatory conditions that enhance germination efficiency.

Author's Contributions

The author conducted the experimental work, performed data collection and analysis, and prepared the manuscript. The study design, interpretation of results, and final revision of the manuscript were completed under the supervision and guidance of the academic advisors.

Conflict of Interest

The author declares no conflicts of interest related to the research, authorship, or publication of this article.

Generative AI and AI-assisted technology statement.

Generative AI tools were used exclusively to improve the clarity and language quality of the manuscript. No AI tools were involved in generating, analyzing, or interpreting the research data. The author is fully responsible for the scientific content and conclusions.

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