


ENHANCING GERMINATION AND SEEDLING VIGOR IN NIGELLA SATIVA: THE IMPACT OF NANO IRON OXIDE AND PHOSPHORUS PRIMING

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ABSTRACT

Nigella sativa, commonly known as black cumin, is known for its health benefits due to its rich content of compounds like thymoquinone. This study explored the effects of various seed priming methods on *Nigella sativa* seeds, including control (unprimed), distilled water, nano iron oxide (Fe_2O_3), phosphorus (P), and a combination of Fe_2O_3 and P. We assessed how these treatments influenced several key aspects of seed germination: germination rate (GR), mean germination time (MGT), germination index (GI), coefficient of velocity of germination (CVG), and vigor index (VI).

Our results showed that seeds treated with Fe_2O_3 nanoparticles had the highest germination rate at 93.5% and germinated faster than seeds from other treatments. This suggests that Fe_2O_3 nanoparticles effectively speed up the germination process. However, despite the improved germination rate, the seeds treated with Fe_2O_3 did not exhibit the highest seedling vigor; the control seeds had the highest vigor index, indicating that while Fe_2O_3 accelerates germination, it does not enhance seedling health as much as the untreated seeds. Additionally, there were no significant differences in the germination index and coefficient of velocity of germination between the treatments, suggesting these parameters were less influenced by the priming methods.

In summary, nano-priming with Fe_2O_3 is effective for speeding up seed germination but does not necessarily improve seedling vigor. These findings provide valuable insights into how different priming techniques can be optimized to enhance seed performance and plant growth under various conditions.

Keywords: *Nigella Sativa*, Seed Priming, Nano Iron Oxide (Fe_2O_3), Priming Treatments

1. INTRODUCTION

1.1. BLACK CUMIN INTRODUCTION

Nigella sativa, commonly known as black cumin or black seed, is an annual flowering plant belonging to the Ranunculaceae family. It has been utilized for centuries in traditional medicine, particularly in the Middle East and South Asia, due to its diverse therapeutic properties. The seeds of *Nigella sativa* contain a rich array of bioactive compounds, including thymoquinone, which is recognized for its antioxidant, anti-inflammatory, and antimicrobial activities [Abedi et al. \(2017\)](#), [Farhangi et al. \(2016\)](#). The pharmacological potential of *Nigella sativa* extends to

various health benefits, including its role in managing conditions such as diabetes, hypertension, and infections [Farhangi et al. \(2016\)](#), [Sandhya & Gomathi \(2020\)](#)

The germination of *Nigella sativa* seeds is influenced by several environmental factors, including temperature, moisture, and the presence of growth-promoting substances. Priming is a pre-sowing treatment that enhances seed germination and seedling vigor by inducing physiological changes within the seeds. Research has demonstrated that priming methods, such as soaking seeds in distilled water or osmotic solutions like polyethylene glycol and mannitol, can significantly improve germination rates and enhance the thymoquinone content in *Nigella sativa* seeds [Rezaei-Chiyaneh et al. \(2018\)](#), [Sandhya & Gomathi \(2020\)](#).

Priming is a method that has gained traction in recent years, as it prepares seeds for germination by initiating physiological processes without allowing actual germination to occur. This technique can lead to faster and more uniform germination, improved seedling establishment, and enhanced resistance to biotic and abiotic stresses [Suri et al. \(2019\)](#), [Shrestha et al. \(2020\)](#). Seed priming is a pre-sowing treatment that enhances seed germination and seedling vigor by exposing seeds to a controlled hydration process, allowing them to initiate early metabolic activities without actual germination. Priming techniques, such as hydropriming (soaking seeds in water), osmo-priming (using osmotic solutions like polyethylene glycol), and hormone priming (using plant growth regulators), have been shown to improve the germination performance of *Nigella sativa*, particularly under stress conditions. These priming methods help to overcome dormancy, enhance water uptake, and activate enzymes involved in germination, leading to faster and more uniform emergence, even under suboptimal conditions. In addition various studies have indicated that priming *Nigella sativa* seeds can significantly enhance their germination parameters, such as germination percentage and speed, thereby contributing to more robust plant growth and development [El-Kenany et al. \(2014\)](#), [Suri et al. \(2019\)](#). The application of osmotic solutions, such as polyethylene glycol or salt solutions, during the priming process has been found to be particularly effective in mitigating stress conditions that seeds may encounter during germination [Suri et al. \(2019\)](#), [Shrestha et al. \(2020\)](#).

Research on the germination parameters and priming of *Nigella sativa* is crucial for optimizing its cultivation, especially in regions prone to environmental stressors. Understanding these factors can help farmers and growers improve seedling establishment, maximize yields, and sustain the medicinal and economic value of this important crop. Furthermore, the application of priming techniques has been shown to improve the overall health and yield of *Nigella sativa* crops. By optimizing germination parameters, farmers can achieve more uniform and robust plant stands, which are crucial for maximizing the medicinal properties of the harvested seeds [Rezaei-Chiyaneh et al. \(2018\)](#), [Yimer et al. \(2019\)](#). The integration of such practices in agricultural settings not only contributes to better crop performance but also supports the sustainable cultivation of this valuable medicinal plant

Priming is an innovative agricultural technique that enhances seed germination and seedling vigor by pre-conditioning seeds before planting. Among the various priming methods, nano-priming has emerged as a particularly effective approach, utilizing nanoparticles to improve seed performance under various environmental stresses. This technique involves the application of nanomaterials, such as metal oxides and phosphates, to seeds, which can significantly enhance their germination rates, growth, and resistance to abiotic stresses like drought and salinity [Rahimi et al. \(2016\)](#), [Nile et al. \(2022\)](#), [Acharya et al. \(2020\)](#)

Recent studies have demonstrated that nano-priming can lead to improved water uptake and metabolic activity in seeds. For instance, the use of cobalt ferrite nanoparticles in the nano-priming of *Phaseolus vulgaris* seeds resulted in a notable increase in the mineral composition of progeny seeds, indicating enhanced nutrient uptake and utilization [Perea-Vélez et al. \(2024\)](#). Nano-Priming of *Phaseolus Vulgaris* oti Cultivar with Cobalt Ferrite Nanoparticles Enhances the Mineral Composition of Progeny Seeds. Similarly, the application of zinc oxide nanoparticles has been shown to increase water absorption in wheat grains, enhancing germination rates compared to traditional hydro-priming methods [Perea-Vélez et al. \(2024\)](#), [Nile et al. \(2022\)](#). The physiological mechanisms underlying the benefits of nano-priming are multifaceted. Nanoparticles can penetrate seed coats more effectively than conventional priming agents, leading to improved hydration and nutrient absorption [Rahimi et al. \(2016\)](#) , [Korishettar et al. \(2017\)](#) Additionally, nano-priming has been associated with the modulation of reactive oxygen species (ROS) homeostasis and the enhancement of enzymatic activities, such as α -amylase, which plays a vital role in starch metabolism during germination [Khan et al. \(2021\)](#). This modulation helps in maintaining cellular integrity and promoting seedling vigor under stress conditions, such as salinity and drought [Khan et al. \(2021\)](#). Furthermore, the application of titanium dioxide (TiO₂) nanoparticles in seed priming has been linked to enhanced phytohormonal activity, which can further promote seed germination and growth. The integration of nanoparticles into seed priming protocols not only improves germination rates but also enhances the overall quality and yield of crops, making it a promising strategy for sustainable agriculture [Nile et al. \(2022\)](#) , [Acharya et al. \(2020\)](#) In the context of *Nigella sativa*, the application of nano-priming techniques could potentially enhance the germination and growth of this medicinal plant, which is already recognized for its numerous health benefits. The use of nanoparticles could improve the bioavailability of essential nutrients and enhance the plant's resilience to environmental stresses, thereby maximizing its therapeutic potential [Oyero et al. \(2016\)](#). In conclusion, nano-priming represents a cutting-edge approach in seed technology that holds great promise for improving agricultural productivity and sustainability. By leveraging the unique properties of nanoparticles, this technique can enhance seed performance, particularly in challenging growing conditions, and contribute to the cultivation of valuable crops like *Nigella sativa*.

Nano iron oxide (Fe₂O₃-NP) and phosphorus (P) are two important agents that have shown promise in priming applications for various crops, including *Nigella sativa*. Nano iron oxide particles possess high reactivity, surface area, and bioavailability, which make them effective in enhancing seed vigor and germination. When applied as a priming agent, Fe₂O₃-NP can improve the availability of iron to seeds, which is a crucial micronutrient for numerous physiological and biochemical processes, including chlorophyll synthesis, enzyme activation, and electron transport during photosynthesis. In *Nigella sativa*, priming with nano iron oxide has the potential to improve germination rates and seedling vigor, especially under adverse conditions such as salinity or drought stress. The application of Fe₂O₃-NP can reduce oxidative stress during germination by enhancing the antioxidant defense system in the seeds, thereby promoting more robust seedling development.

Phosphorus is another essential nutrient involved in key metabolic processes, including energy transfer, nucleic acid synthesis, and root development. Phosphorus priming is particularly beneficial in improving early root growth and establishment, as it enhances the availability of phosphorus during germination and the initial stages of plant development. For *Nigella sativa*, phosphorus priming can stimulate faster and more uniform germination by improving the metabolic readiness of the

seed. Moreover, phosphorus plays a vital role in improving drought tolerance and promoting root system development, which is critical for water and nutrient uptake in the early growth stages of the plant.

Combining nano iron oxide and phosphorus in priming treatments can have synergistic effects, enhancing both the physiological and biochemical performance of *Nigella sativa* seeds under stress conditions. The dual application of these agents could help alleviate oxidative damage, promote nutrient uptake, and improve overall seedling vigor, leading to enhanced crop establishment and yield, particularly in regions where environmental stress is prevalent.

The objective of this study is to evaluate the effects of various seed priming treatments, including control (unprimed), distilled water, nano iron oxide (Fe_2O_3), phosphorus (P), and the combination of nano iron oxide and phosphorus ($\text{Fe}_2\text{O}_3 + \text{P}$), on the germination performance of *Nigella sativa*. By investigating these priming methods, the study aims to determine their potential to enhance germination rates, seedling vigor, and, particularly under challenging environmental conditions. This research seeks to identify the most effective priming technique for improving the establishment and overall yield of *Nigella sativa* in agricultural systems.

2. MATERIAL AND METHOD

This study examines the impact of various priming treatments on *Nigella sativa* L. seeds under controlled laboratory conditions. The seeds were subjected to germination tests, placed on four layers of moistened filter paper, and incubated in darkness at a constant temperature of 22°C. Germination was assessed by the emergence of a 1 mm radicle and monitored over a 10-day period. No signs of microbial contamination were observed during the experiment [Kamal et al. \(2010\)](#).

For priming, there are five applications; Control is no priming, distilled water, nano iron oxide (Fe_2O_3), phosphorus (P), and the combination of nano iron oxide and phosphorus ($\text{Fe}_2\text{O}_3 + \text{P}$). Seeds were soaked in control (unprimed), distilled water, nano iron oxide (Fe_2O_3), phosphorus (P), and the combination of nano iron oxide and phosphorus ($\text{Fe}_2\text{O}_3 + \text{P}$), distilled water (dH₂O) [Basra et al. \(2004\)](#). The experimental design was arranged in a completely randomized design with 4 replications of 25 seeds in the germination stage.

A solution of iron oxide nanoparticles ($\text{Fe}_2\text{O}_3\text{NPs}$) was prepared by dissolving 10 mg of the material in 100 mL of water. Similarly, a phosphorus (P) solution was made by dissolving 0.3 g of phosphorus in 1 liter of water. For each treatment, 20 grams of seeds were measured out, with four replicates per treatment. The volume of solution used was 1.5 times the weight of the seeds in the container. Seeds were submerged in 250 mL of the prepared solution for 8 hours, ensuring they were fully covered, and then dried. The seeds were placed in 10 × 10 cm plastic boxes lined with blotting paper. After germination started at 36 hours, an additional 6 mL of the solution was added to each replicate.

Germination Rate (%): It is calculated as Scott et al., 1984.

$$GR = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} * 100$$

Germination Index: The germination index is calculated by dividing the number of germinated seeds by the number of days to the first count, and adding that to the number of germinated seeds divided by the number of days to the final count [Benech Arnold et al. \(1991\)](#).

Mean germination time (Day): It is calculated as Ellis & Roberts, 1981.

$$MGT = \frac{\sum(Dn)}{\sum n}$$

n: number of seeds germinated on day D,

D: number of days counted from the beginning of the germination the lightest seeds,

Coefficient of Velocity of Germination (CVG): CVG indicates the rate at which seeds germinate over a specific period. It is a measure of how quickly the germination process occurs. A higher CVG value indicates faster germination, while a lower CVG value suggests slower germination. Coefficient of velocity of germination is an index for germination speed [Maguire \(1962\)](#).

$$CVG = \frac{G1 + G2 + G3 \dots + Gn}{(1 \times G1) + (2 \times G2) + \dots (n \times Gn)} (\text{seed day})^{-1}$$

G = number of seeds germinated on day t

t (1, 2,...n) = days from the start of the test

Germination Vigor Index (Vi): The Germination Vigor Index (Vi) assesses the vigor or strength of seed germination by incorporating both the speed of germination and the seedling growth. It is a more comprehensive measure of seed vigor than simple germination percentage.

$$Vi = (\text{Germination Percentage}) (\text{Mean Seedling Height})$$

Statistical analyses of the experimental data were performed using the JMP software package. Variance analysis (ANOVA) was conducted to assess the differences among the examined traits, and mean comparisons were determined using the Least Significant Difference (LSD) test at the 5% and 1% significance levels.

3. RESULTS AND DISCUSSION

The analysis of variance demonstrated that distinct priming treatments exerted a notable influence on multiple germination parameters of *Nigella sativa* seeds. The germination rate (GR) was found to be significantly influenced by the priming treatments ($p < 0.05$), with a mean square value of 57.3, indicating substantial variation among the treatments. In contrast, the mean germination time (MGT) was not found to be significantly affected by the treatments, as indicated by a non-significant mean square value. Similarly, no significant differences were observed for the germination index (GI) and the coefficient of velocity of germination (CVG) across treatments [Table 1](#)

However, the vigor index (VI) demonstrated a statistically significant response to the priming treatments ($p < 0.05$), with a mean square value of 0.448, indicating that priming may enhance seed vigor under specific conditions. The LSD 0.05 value for GR was calculated as 4.98, and for VI as 0.449, confirming the presence of

statistically significant differences between the treatments for these parameters. Furthermore, replication effects were also significant for GR and MGT, indicating some variation between different trials of the experiment [Table 1](#)

Table 1

Table 1 Analysis of Variance for the Effect of Different Priming Treatments (Control (Unprimed), Distilled Water, Fe₂O₃Nps, P and the Combination of Fe₂O₃Nps + P) on Some Germination Parameters of *Nagilla Sativa* Seeds

Source of Variance	df	Mean Square				
		GR	MGT	GI	CVG	VI
Rep.	3	48*	0,1256*	0,0799	1,679	0,1804
Treatment	4	57,3*	0,078ns	0,6427ns	2,04ns	0,448*
Error	12	10,5	0,25	0,0877	1,06	0,085
LSD _{0,05 (T)}		4.98	0,243	ns	ns	0,449

GR: Germination rate, MGT: Mean germination time, GI:Germination index, CVG: Coefficient of Velocity of Germination, Coefficient of Velocity of Germination, VI: Germination Vigor Index

The priming treatments significantly impacted the germination rate (GR) of *Nigella sativa* seeds. Seeds treated with Fe₂O₃ nanoparticles (Fe) had the highest germination rate (93.5), standing out significantly compared to the control and other treatments, as shown by the LSD analysis (group A). Meanwhile, the control, distilled water (dH₂O), Fe + P, and P treatments had lower but similar germination rates, all grouped under letter B. There were also clear differences in mean germination time (MGT) across treatments. Seeds treated with Fe germinated the fastest, with a significantly shorter MGT than the control and other treatments. In contrast, seeds in the control group had the slowest germination, taking 4.29 days on average, and were placed in LSD group A. The vigor index (VI) was also notably affected by the treatments. Seeds from the control group had the highest vigor index (3.41), significantly surpassing all other treatments, which were grouped under letter B. This indicates that the control treatment led to stronger seedlings compared to the others, including dH₂O, Fe, Fe + P, and P, which showed reduced vigor. However, no significant differences were found between treatments for the germination index (GI) and the coefficient of velocity of germination (CVG), based on the ANOVA results. Although some numerical variations were observed, the treatments did not have a statistically meaningful effect on these parameters [Table 2](#)

Table 2

Table 2 The Effects of Different Priming Treatments (Control (Unprimed), Distilled Water, Fe₂O₃Nps, P and the Combination of Fe₂O₃Nps + P) on Some Germination Parameters of *Nagilla Sativa* Seeds

Priming Treatments	GR	MGT	GI	CVG	VI
Control	88,00 B	4,29 A	5,33	23,29	3,41 A
dH ₂ O	85,00 B	4,07 AB	5,55	24,58	2,81 B
Fe	93,50 A	3,85 B	5,55	25,013	2,74 B
Fe+P	84,50 B	4,18 A	5,49	23,85	2,50 B
P	85,00 B	4,25 A	5,28	23,58	2,89 B

GR: Germination rate, MGT: Mean germination time, GI: Germination index, CVG: Coefficient of Velocity of Germination, Coefficient of Velocity of Germination, VI: Germination Vigor Index

The results of this study reveal that different priming treatments have a significant impact on various germination parameters of *Nigella sativa* seeds. Among these treatments, Fe₂O₃ nanoparticles (Fe) stood out, boosting the germination rate (GR) to 93.5, which was notably higher than the other treatments. This finding supports earlier research by [Nair et al. \(2013\)](#), who found that nanoparticles can enhance seed growth and development due to their ability to penetrate seed tissues and improve nutrient uptake.

Seeds treated with Fe also had a significantly shorter mean germination time (MGT), meaning they sprouted faster. This is in line with [Zhao et al. \(2014\)](#), who observed that nanoparticles can speed up germination by accelerating the metabolic processes within seeds. In contrast, seeds from the control group had the slowest germination, taking the longest time to sprout, which indicates that Fe treatment effectively speeds up the germination process.

Interestingly, while Fe improved both the germination rate and speed, it did not enhance seedling vigor as much as the control treatment. The control group had the highest vigor index (3.41), suggesting that although Fe treatment boosts germination metrics, it doesn't necessarily translate into stronger seedlings. This is consistent with the work of [Ashraf & Foolad \(2005\)](#), who pointed out that improvements in germination rate do not always lead to better seedling health.

Additionally, no significant differences were found in the germination index (GI) and the coefficient of velocity of germination (CVG) between treatments. This suggests that while some priming methods affected germination rate and speed, they didn't make a substantial difference in overall germination efficiency or uniformity. [Bewley and Black \(1994\)](#) noted similar findings, indicating that certain parameters might be less sensitive to some treatments compared to others.

The significant replication effects observed for GR and MGT underline the need for consistent experimental conditions. Variations between trials can influence results, as [Lutts, S., et al. \(2016\)](#) and [Forti \(2020\)](#) highlighted in his review on seed testing methods. It's crucial to maintain uniform conditions to accurately assess the impact of different priming treatments on seed performance.

At the end of study, Fe₂O₃ nanoparticles were effective in improving both germination rate and speed, but the control treatment resulted in better seedling vigor. The other treatments didn't significantly affect the germination index or coefficient of velocity of germination. This adds to our understanding of how various priming methods influence seed germination and seedling growth.

4. CONCLUSION

The findings of this study indicate that priming treatments exert a considerable impact on the germination and early growth of *Nigella sativa* seeds. Among the treatments tested, the application of Fe₂O₃ nanoparticles (Fe) notably enhanced the germination rate and reduced the mean germination time, indicating a faster and more effective sprouting process compared to other treatments. However, while Fe improved these parameters, it did not enhance seedling vigor to the same extent as the control treatment, which produced the strongest seedlings.

The findings indicate that Fe₂O₃ nanoparticles have the potential to be a valuable tool for improving seed germination speed; however, they may not always result in enhanced seedling health. Conversely, the control treatment exhibited

superior seedling vigor, indicating that it may be more efficacious for applications where seedling strength is a critical factor. The absence of notable differences in the germination index (GI) and coefficient of velocity of germination (CVG) across treatments indicates that these parameters are less responsive to the priming methods employed in this study. This emphasises the intricate nature of seed response to diverse priming treatments and reinforces the necessity of assessing a multitude of parameters when evaluating seed performance. In Conclusion, Future studies should also investigate the underlying physiological mechanisms that contribute to the observed effects, as well as the potential applications of these findings in agricultural practices aimed at optimizing seed performance and crop yield.

5. AUTHOR CONTRIBUTIONS

Hatice Kübra Gören: writing–original draft, writing–review and editing, methodology, conceptualization. Öner Canavar: supervision, Uğur Tan: writing–review and editing, visualization, formal analysis.

6. ETHICS STATEMENT

Ethical approval was not sought for this study, as it did not involve human subjects or patient data. Consent No patient consent was required for this study, as it did not involve human subjects or patient data.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest. Data Availability Statement The data used in this study are available upon reasonable request from the corresponding author

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