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Enhancing the Establishment of Black Cumin (*Nigella sativa* L.): The Role of Seed Improvement Techniques and Cultivation Substrate

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ABSTRACT

Seed coating is an economically viable method for seed enhancement, and biological seed treatments in the form of coating can substantially improve plant performance. This study, conducted in Bardaskan city, Razavi Khorasan province, Iran, aimed to evaluate the effects of seed enhancement and substrate on black cumin (*Nigella sativa*) and employed a two-factor factorial design based on a completely randomized design (CRD) and included three replications. The first factor included four treatments: control (field soil), field soil + farmyard manure (25% v/v), field soil + chemical fertilizers (ammonium sulfate as nitrogen source and triple superphosphate as phosphorus source), and field soil + biofertilizers. The second factor (seed pretreatment) consisted of four treatments: uncoated seeds (control), micronutrient-coated seeds, *Azotobacter chroococcum* -inoculated seeds, and combined micronutrient and *A. chroococcum* coating. The optimal treatment (*A. chroococcum*+ micronutrients under field soil + biofertilizer) resulted in 80% seed emergence, shoot length (5.08 cm), root length (3.27 cm), and a seedling vigor index of 6.68. This treatment also reduced oxidative stress markers, with hydrogen peroxide and malondialdehyde contents decreasing to 0.2 μmol g^{-1} FW and 15.24 nmol g^{-1} FW, respectively, while enhancing antioxidant capacity (86.6%) and phenolic content (87.4 mg GAE g^{-1} FW). Correlation and principal component analyses revealed a clear inverse relationship between oxidative stress markers and growth parameters, indicating that growth enhancement was achieved through increasing antioxidant capacity and phenolic content, and reducing oxidative stress. The substrate amended with biofertilizer consistently performed better than those treated with chemical fertilizers, emphasizing the significance of biological complexity rather than just focusing on nutrient supplementation. These findings show that integrated bio-fertilization strategies can fundamentally alter seedling physiology, offering a sustainable approach for medicinal plant cultivation that simultaneously enhances productivity, antioxidant properties, and environmental sustainability.

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1. Introduction

Black cumin (*Nigella sativa* L.) is an annual herbaceous plant whose medicinal properties are primarily attributed to its key bioactive compound, thymoquinone, which exhibits significant antioxidant, anti-inflammatory, and therapeutic effects (Yimer *et al*., 2019). Black cumin is cultivated in various climates across Iranian provinces, including Khorasan and Isfahan. It relies on local landraces that have historically contributed to crop adaptation and agricultural resilience since the dawn of agriculture. In Iran, the highest reported seed yields can reach 186.64 g m^{-2} under optimized conditions (Hosseini *et al*., 2018). In contrast, global averages in major producing countries like Turkey range from 458.9 to 790.3 kg ha^{-1} (Kara *et al*., 2015). The economic significance of black cumin is further illustrated by its seed oil market, which was valued at USD 20.94 million in 2023 and is projected to grow to USD 36.59 million by 2030, reflecting a compound annual growth rate (CAGR) of

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8.3%. This growth is driven by increasing demand in the health and food sectors (MMR, 2025). These factors highlight the potential for improved cultivation practices to enhance productivity and commercial value.

The cultivation substrate critically influences plant production by fulfilling essential requirements such as water and nutrient availability, root zone oxygenation, and gas exchange between roots and atmosphere (Samal *et al*., 2019). To address these requirements, early-stage nutrient deficiencies and poor substrate conditions often hinder uniform emergence and vigorous seedling growth in black cumin, reducing overall yield potential and increasing susceptibility to environmental stresses (Ekren *et al*., 2023). For successful black cumin cultivation, selecting a substrate with adequate porosity and water-holding capacity, combined with supplementation using at least 50% Hoagland solution, enhances seedling establishment and promotes optimal capsule development (Weiss *et al*., 2021). Despite such substrate optimization, nutrient deficiency during early growth remains a major constraint, limiting vegetative development and reducing competitive ability against weeds through slower growth and reduced canopy expansion (Wilson and Roberts, 2014). Germination represents the primary developmental stage in seed growth and development, holding significant biological importance. In addition to germination capacity, emergence rate and uniformity serve as critical indicators of seed quality (Forti *et al*., 2020).

Seed coating has become one of the most cost-effective methods for enhancing seed performance. This technique involves applying specialized materials to seeds with specific objectives, including the promotion of seedling growth, protection against insects and rodents, and mitigation of detrimental effects caused by abiotic stresses (Piri *et al*., 2019; Paravar *et al*., 2023).

In particular, such coatings serve as efficient carriers for both micronutrients and biological agents to overcome early nutrient limitations and environmental stresses (Javed *et al*., 2022). More precisely, seed coating aims to utilize various materials such as fungicides, safening agents, micronutrients, and other growth-promoting compounds that directly interact with the seed (Hosseini-Moghddam *et al*., 2024).

Furthermore, beneficial plant microorganisms, including growth-promoting bacteria and fungi, demonstrate the capacity to enhance seed germination, improve crop yield, increase plant tolerance to biotic stresses (pathogens and pests), abiotic stresses (salinity, drought, heavy metals), reduce dependence on chemical fertilizers and their associated adverse environmental impacts (Piri *et al*., 2019). Accordingly, integrating plant growth-promoting microorganisms with micronutrient seed coatings creates a synergistic biological fertilizer strategy that significantly enhances plant performance by addressing early nutrient limitations and cultivation challenges (Fazeli-Nasab *et al*., 2025; Guo *et al*., 2025).

Current agricultural practices utilize three primary application methods for biofertilizers and micronutrients: soil incorporation, foliar application, and seed treatment. Comparative analysis reveals seed treatment is the most cost-effective method, requiring lower amounts of micronutrients than soil application. It also simplifies application protocols, enhances seedling vigor and establishment, and provides significant cost-benefit advantages. Given the higher costs of soil incorporation and foliar application, seed treatment remains the most economically favorable option (Ammar *et al*., 2023; Singh *et al*., 2023).

Despite extensive documentation of black cumin medicinal properties and agronomic requirements, critical knowledge gaps persist regarding: 1) Optimal seed treatment protocols combining biofertilizers and micronutrients for enhanced black cumin production. 2) Synergistic effects of plant growth-promoting microorganisms with micronutrient coatings under varying cultivation conditions. 3) Standardized formulations for black cumin -specific seed coatings addressing both biotic and abiotic stress mitigation. 4) Economic feasibility analyses of advanced seed treatments versus conventional fertilization methods. These findings underscore that the selection of appropriate cultivation substrate and nutritional supplements constitutes fundamental prerequisites for economically viable black cumin cultivation.

This study aims to evaluate the combined effects of *A.chroococcum* -micronutrient seed coating and biofertilizer-amended substrates on seedling emergence, growth parameters, oxidative stress markers, and antioxidant capacity in *N. sativa*.

2. Materials and methods

2.1. Plant materials and experimental site

The experiment was conducted during the 2020-2021 growing season at the research greenhouse of the Bardaskan Agricultural Research Center, Bardaskan County, Razavi Khorasan province, Iran (56°14'E longitude, 58°15'N latitude; Altitude: 1450 m above sea level).

2.2. Experimental design

This study employed a two-factor factorial arrangement within a completely randomized design (CRD) with three replications to evaluate the effects of cultivation substrates and seed pretreatment on germination indices and growth parameters of black cumin plant. Factor A: Cultivation substrates (4 levels): 1) control (Field soil), 2) field soil + farmyard manure (25% v/v), 3) field soil + chemical fertilizers: Ammonium sulfate (N source; 50 kg ha^{-1}), triple superphosphate (P source; 40 kg ha^{-1}), 4) field soil + biofertilizers (manufacturer's recommended dosage): *Azotobacter chroococcum* (10^8 CFU g^{-1}) Phosphate solubilizing bacteria (Rocha *et al*., 2019; Paravar *et al*., 2023). Factor B: seed treatments (4 levels): 1) untreated or control, 2) micronutrient coating, 3) *A. chroococcum* inoculation (10^8 CFU mL^{-1}), 4) combined treatment: micronutrient coating + *A. chroococcum*. For the combined treatment, seeds were first soaked in gum arabic, then inoculated with *Azotobacter* bacteria. Also, before sowing, the seeds were inoculated with micronutrients in the ratio of 100 grams of seeds to 7 grams of micronutrients, and the inoculation method was the same as inoculating the seeds with *A. chroococcum* bacteria. Then the seeds were immediately sown. The composition of micronutrients included: Mo, Zn, Mn, Cu, Fe and B_2O_5, and their contents were 0.2, 0.7, 10.2, 6.7, 99.60 and 2.12%, respectively (Mirshekari *et al*., 2010). The physicochemical properties of the soil are presented in Table 1.

Table 1. Characteristics of farm soil at a depth of 0 to 30 cm.

pH	EC (ds m^{-1})	K (mg kg^{-1})	P	OM* (%)	Sand	Silt	Clay
8.1	2.2	172	6.4	0.25	48	36	14

*OM: Organic matter

2.3. Data collection and biochemical measurement

Seedling parameters were recorded daily for 14 days post-sowing: Seedling emergence percentage and growth indices included shoot and root length (cm), and seedling vigor index.

$$\text{Seedling Emergence (\%)} = \frac{\text{Total Number of Germinated Seed}}{\text{Total Number of Seeds}} \times 100 \quad (1)$$

$$\text{Seedling Vigor Index} = \text{Seedling Emergence (\%)} \times \text{Seedling Length} \quad (2)$$

Then, after 14 days of cultivation in pots, the seedlings were separated and placed in a refrigerator at -18°C to measure MDA content, H_2O_2 content, total antioxidant capacity, and total phenolic content.

2.4. Malondialdehyde assay

Lipid peroxidation was assessed by quantifying malondialdehyde (MDA) levels following the procedure outlined by Hichem and Mounir (2009). Briefly, 0.15 g of fresh plant tissue was homogenized in 20% trichloroacetic acid (TCA) and then centrifuged at 20,000 g for 20 minutes. The resulting supernatant was incubated with 0.5% thiobarbituric acid (TBA) at 95°C for 30 minutes. After cooling and subsequent centrifugation, absorbance was measured at 532 nm and 600 nm using a spectrophotometer (UV-160A Shimadzu, Japan). The MDA concentration was determined using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed in nanomoles per gram of fresh weight.

2.5. Hydrogen peroxide (H_2O_2) content

The hydrogen peroxide content was measured according to the method described by Velikova et al. (2000). Fresh tissue (0.15 g) was homogenized in 0.2% TCA and centrifuged at 20,000 × g for 20 minutes at 4°C. Then, 500 µL of the supernatant was mixed with 500 µL of 10 mM phosphate buffer and 500 µL of 1 M potassium iodide solution. The absorbance was recorded at 390 nm using a spectrophotometer (UV-160A Shimadzu, Japan), and the H_2O_2 concentration was calculated using a standard curve, expressed as micromoles per gram of fresh weight.

2.6. Total antioxidant capacity assay

The free radical scavenging activity of the extracts was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, as per Hichem and Mounir (2009). A 300 µL aliquot of the methanolic extract was combined with 2 mL of 0.1 mM DPPH solution and incubated in the dark for 20 minutes. Absorbance was measured at 517 nm using a spectrophotometer (UV-160A Shimadzu, Japan), and the percentage of DPPH radical scavenging activity was determined.

2.7. Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu method (Bao *et al*., 2005). A 100 µL aliquot of the methanolic extract was mixed with 200 µL of Folin–Ciocalteu reagent and incubated for 5 minutes. Then, 800 µL of 7.5% sodium bicarbonate solution was added, and the mixture was allowed to stand for 1 hour. Absorbance was measured at 765 nm using a spectrophotometer (UV-160A Shimadzu, Japan), and the total phenolic content was expressed as milligrams of gallic acid equivalent per gram of fresh weight (mg GAE g^{-1} FW).

2.8. Statistical analysis

Data were subjected to two-way ANOVA using SAS (V 9.4) at the 1% significance level. Mean comparisons employed Tukey's HSD test (α=0.05). Assumptions of normality (Shapiro-Wilk) and homogeneity (Levene's test) were verified.

3. Results and discussion

3.1. Growth parameters

According to the ANOVA results, the interaction between cultivation substrates and seed pretreatment significantly influenced all growth parameters at the 1% probability level (Table 2). The combination of *A. chroococcum* and micronutrients as seed pretreatment, along with field soil and biofertilizer as the cultivation substrate, produced the highest values: seed emergence percentage (SEP) of 80% (Fig. 1), shoot length of 5.08 cm (Fig. 2), root length of 3.27 cm (statistically comparable to *A. chroococcum* alone at 3.2 cm) (Fig. 3), seedling length of 8.35 cm (Fig. 4), and seedling vigor index (SVI) of 6.68 (Fig. 5). In contrast, uncoated seeds in field soil exhibited the lowest performance across all indices: SEP of 11.66%, shoot length of 0.19 cm, root length of 0.11 cm, seedling length of 0.31 cm, and SVI of 0.036 (Figs. 1–5).

Table 2. The results of the analysis of variance of the effect of cultivation substrates and seed pretreatments on the seedling indices of black cumin

S.O.V	df	Mean square				
		Seedling emergence	Shoot length	Root length	Seedling length	Seedling vigor index
Cultivation substrates (A)	3	2025.85**	1.16**	1.12**	4.50**	33872.11**
Seed pretreatment (B)	3	2157.44**	2.68**	2.16**	8.99**	61334.25**
A × B	9	970.52**	1.16**	1.85**	5.48**	34571.24**
Error	32	68.99	0.09	0.09	0.26	2336.80
C.V. (%)	-	20.20	25.6	21.40	22.24	24.76

* and ** are significant at 5 and 1 percent probability levels, respectively

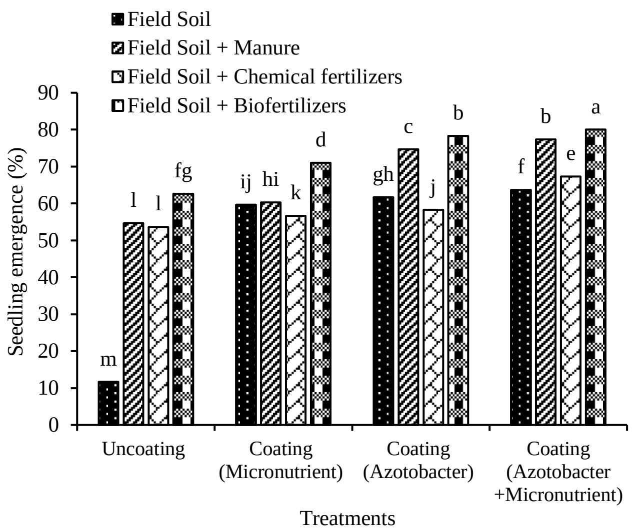

Figure 1. Mean comparison of the effect of seed pretreatment on seedling emergence percentage (SEP) of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

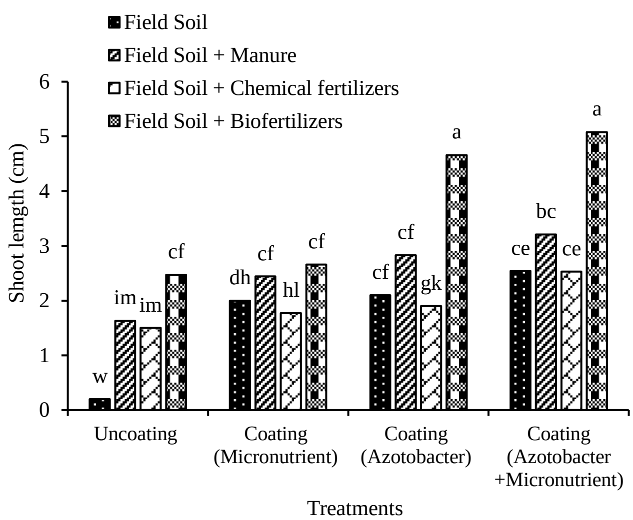

Figure 2. Mean comparison of the effect of seed pretreatment on shoot length of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

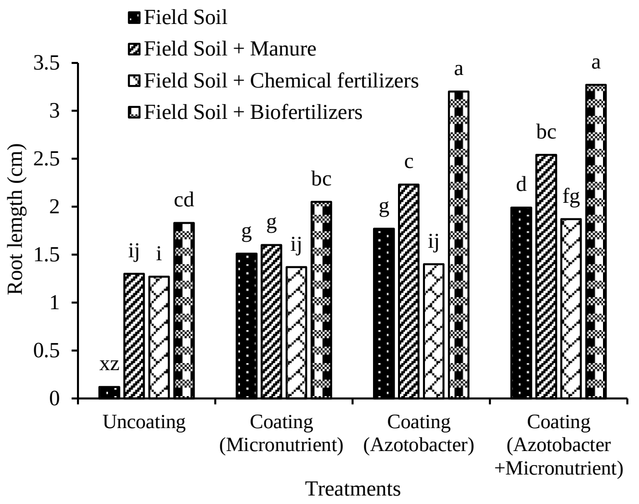

Figure 3. Mean comparison of the effect of seed pretreatment on root length of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

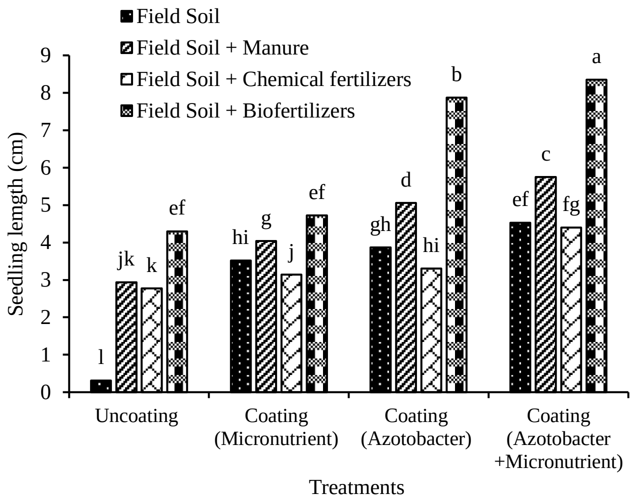

Figure 4. Mean comparison of the effect of seed pretreatment on seedling length of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

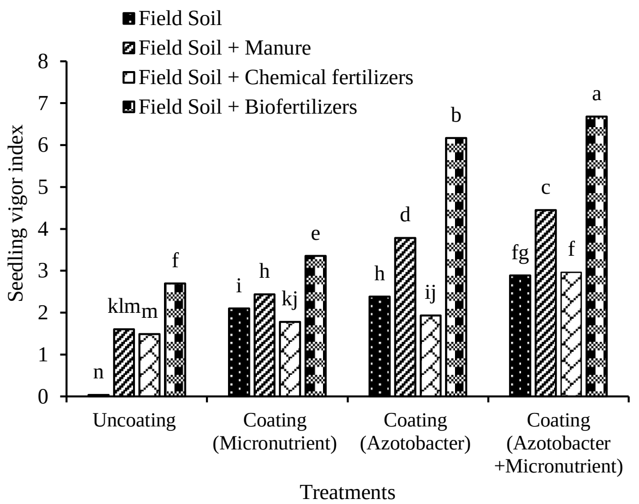

Figure 5. Mean comparison of the effect of seed pretreatment on seedling vigor index (SVI) of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

3.2. Oxidative stress markers

According to the ANOVA results, the simple effects of growth substrates and seed pretreatment interaction significantly influenced H_2O_2 and MDA content, total antioxidant capacity, and phenolic content at the 5% probability level (Table 3).

Table 3. The results of the analysis of variance of the effect of cultivation substrates and seed pretreatments on oxidative stress markers (H_2O_2 and MDA), total antioxidant capacity, and phenolic content of black cumin seedlings.

S.O.V	df	Mean square		
		H_2O_2	MDA	TAC
Cultivation substrates (A)	3	0.155^{*}	92.08^{*}	684.1^{**}
Seed pretreatment (B)	3	0.229^{*}	181.5^{**}	3737.3^{**}
A × B	9	0.015^{*}	21.34^{*}	117.87^{*}
Error	32	0.384	5.02	11.41
C.V. (%)	-	28.19	21.41	18.40

* and ** are significant at 5 and 1 percent probability levels, respectively

An inverse trend was observed for oxidative stress markers. The highest levels of hydrogen peroxide (H_2O_2) were recorded in the uncoated in-field soil treatment, measuring 0.89 μmol g^{-1} FW, and MDA content at 35.06 nmol g^{-1} FW. In contrast, the lowest levels of H_2O_2 at 0.2 nmol g^{-1} FW and MDA at 15.24 nmol g^{-1} FW were found in seeds pretreated with micronutrients + *A. chroococcum* in the soil+ biofertilizer substrate. This optimal treatment consistently reduced both markers across all substrates compared to the controls (Figs. 6 and 7).

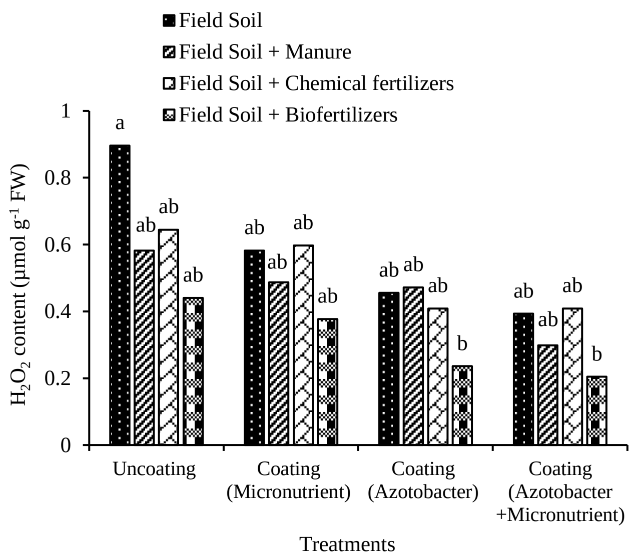

Figure 6. Mean comparison of the effect of seed pretreatment on H_2O_2 content of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

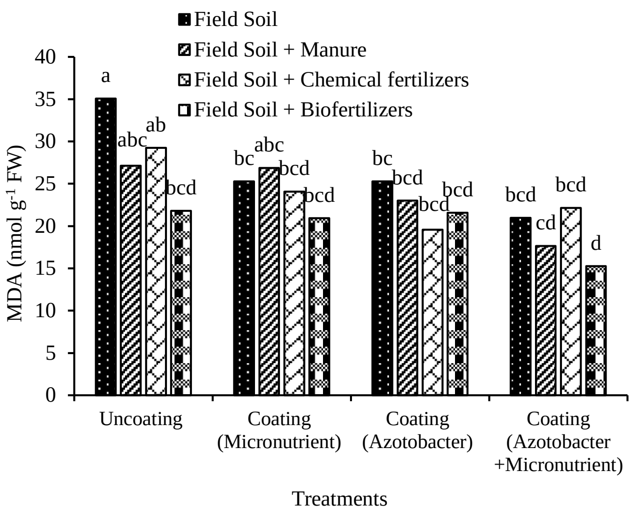

Figure 7. Mean comparison of the effect of seed pretreatment on MDA content of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

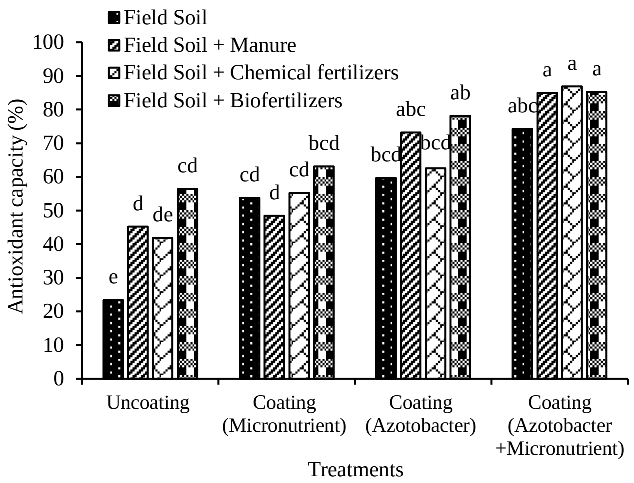

Figure 8. Mean comparison of the effect of seed pretreatment on total antioxidant capacity (TAC) of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

3.3. *Antioxidant capacity and phenolic content*

The highest total antioxidant capacity (86.8%) was achieved with *A. chroococcum* + micronutrients in the chemical fertilizer substrate, statistically on par with the same pretreatment in field soil + manure and field soil + biofertilizer. Phenolic content followed a similar trend, peaking at 87.4 mg GAE g^{-1} FW under chemical fertilization. The lowest values for both parameters (23.37% and 34.01 mg GAE g^{-1} FW) were recorded in uncoated seeds in unamended field soil (Figs. 8 and 9).

The correlation heatmap reveals significant relationships between oxidative markers, secondary metabolites, and growth-related traits during seed germination. H_2O_2 and MDA exhibited a strong positive correlation, reflecting their simultaneous elevation under oxidative stress. Conversely, these two oxidative markers exhibited strong negative correlations with both the total TAC and total phenol content. This inverse relationship suggests that the accumulation of phenolic compounds and the enhancement of overall antioxidant activity play a crucial role in mitigating oxidative damage. Furthermore, all growth-related traits (SEP, Shoot Length, Root Length and SVI) showed strong positive correlations with one another. More critically, these growth parameters were strongly and negatively correlated with H_2O_2 and MDA levels, demonstrating that elevated oxidative stress is directly associated with a significant suppression of seedling growth and development (Fig. 10).

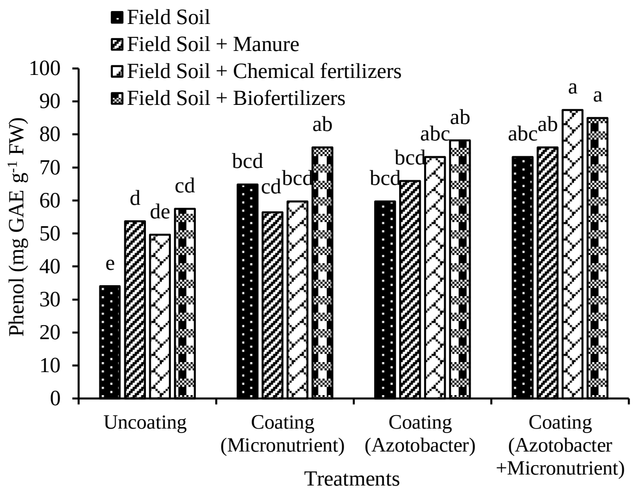

Figure 9. Mean comparison of the effect of seed pretreatment on total phenol content of black cumin under different cultivation substrates. Means followed by the same letter within each column are not significantly different at p≤0.05 according to HSD test.

Figure 10. Heatmap of correlation coefficients between oxidative stress markers (H_2O_2 and MDA), total antioxidant capacity, phenolic content, and seedlings growth indices in black Cumin.

The Principal Component Analysis (PCA) effectively visualizes and confirms the patterns identified by the correlation analysis. Principal Component 1 (PC1) explained 65% of the total variance, while PC2 accounted for 14.2%. The PCA biplot clearly segregates the traits into two distinct groups. The growth parameters (SVI, SEP, Shoot Length, Root Length) cluster together in one quadrant alongside TAC and Phenol, indicating their positive association and shared contribution to the principal components (likely PC1). In stark contrast, H_2O_2 and MDA are positioned in the opposite quadrant, highlighting their strong negative correlation with the first group. This clear spatial separation in the PCA plot underscores a fundamental antagonism between oxidative stress and seedling growth (Fig. 11).

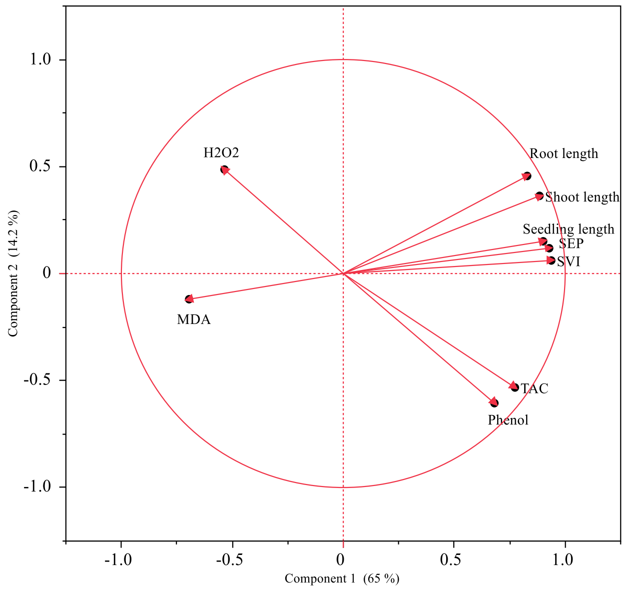

Figure 11. Principal Component Analysis (PCA) biplot displaying the relationships among oxidative stress markers (H_2O_2 and MDA), total antioxidant capacity, phenolic content, and seedlings growth indices in black Cumin.

The establishment of robust seedlings represents a critical physiological determinant in agricultural productivity, particularly for medicinal species such as black cumin. where early developmental vigor significantly influences both biomass accumulation and bioactive compound synthesis. Our comprehensive analysis demonstrates that the synergistic interaction between cultivation substrate composition and seed pretreatment protocols significantly modulates seedling physiology, establishing a trajectory toward enhanced crop establishment and environmental resilience.

The mean comparison of interaction effects between cultivation substrate and seed pretreatment revealed a consistent pattern across all measured growth parameters. The highest values for emergence percentage, shoot length, root length, seedling length, and seedling vigor index were consistently associated with the integrated treatment combining field soil amended with biofertilizer and seed pretreatment utilizing micronutrients with *A. chroococcum* inoculation. In contrast, the most limited growth responses were observed in the non-pretreated seeds cultivated in unamended field soil (Figs. 1-6). Notably, the maximum values obtained for emergence percentage, root length, and seedling length in the combined micronutrient and *A. chroococcum* treatment demonstrated statistical parity with treatments utilizing *Azotobacter* inoculation alone within the biofertilizer-amended substrate (Figs. 2-5), suggesting that the microbial component serves as the primary driver in this synergistic relationship. More critically, these growth parameters were strongly and negatively correlated with H_2O_2 and MDA levels, demonstrating that elevated oxidative stress is directly associated with a significant suppression of seedling growth and development, The results demonstrate that improved seed vigor and seedling growth are intrinsically linked to the reduction of key oxidative stress markers, concurrently with the enhancement of the plant's antioxidant defense system, predominantly represented by total antioxidant capacity and phenolic content (Figs. 10 and 11). The concomitant reduction in MDA and H_2O_2 points to a successful mitigation of oxidative damage by the *A. chroococcum* treatment. The lower MDA content signifies limited lipid peroxidation and, consequently, preserved cell membrane integrity, while the diminished H_2O_2 level reflects an upregulated capacity for reactive oxygen species (ROS) detoxification (Abdel Latef *et al*., 2021).

The mechanistic basis for these observations appears rooted in the fundamental role of nitrogen metabolism in early plant development. As nitrogen constitutes an essential component in germination-related enzymes and influences critical processes including photosynthesis, biochemical interactions, growth duration, and dry matter partitioning (Ali and Elozeiri, 2017), the enhanced nitrogen availability through biological fixation provides a physiological advantage that transcends conventional fertilization approaches.

Our findings clearly demonstrate that field soil amended with chemical nitrogen and phosphorus fertilizers yielded the lowest values across all measured parameters, while the biofertilizer-amended substrate generated the most favorable outcomes. This pronounced disparity underscores the superior efficacy of biological nitrogen fixation mediated by soil microorganisms compared to synthetic fertilizer application, supporting previous research indicating that bacteria, including *Azotobacter* and *Azospirillum*, significantly enhance root development and dry matter accumulation through their nitrogen-fixing capacity and nutrient mobilization abilities (Davar Zareii *et al*., 2014).

The stress-mitigating properties of biofertilizer treatments further substantiate their agricultural value. In agreement with Hoseini et al. (2022), who demonstrated that combining biofertilizer with seed coating mitigates drought-induced reductions in anise seedling growth, our findings confirm that microbial inoculants enhance plant resilience through various mechanisms. The enrichment of cultivation substrates with biofertilizers improves micronutrient availability, particularly zinc, manganese, and iron, thereby accelerating germination kinetics (Motamednezhad *et al*., 2016; Szerement *et al*., 2022) and facilitating more rapid root access to essential nutritional elements (Huang *et al*., 2014). This accelerated nutrient acquisition timeline promotes robust root system development, consequently enhancing water and nutrient uptake efficiency (Seleiman *et al*., 2021), and ultimately increasing overall plant biomass and nitrogen use efficiency (Hamoud *et al*., 2019).

The physiological benefits of microbial inoculation extend to stress tolerance mechanisms, as evidenced by Piri et al. (2021), who observed that despite decreasing germination indices in cumin under escalating osmotic stress, inoculated seeds maintained superior germination percentages and root elongation compared to non-inoculated controls across all stress levels (Gao *et al*., 2020). This enhanced stress resilience aligns with the capacity of biofertilizers to improve soil physicochemical properties, including organic matter content and NPK availability (Gao *et al*., 2020), while simultaneously balancing nutritional elements and producing growth-promoting compounds (Kundan *et al*., 2015). The expansion of root surface area through enhanced root hair development, facilitated by bacterial-derived hormones and macromolecules, significantly increases nutrient interception capacity (Miljakovic *et al*., 2020), creating a positive feedback loop that further supports plant development.

From an ecological standpoint, transitioning from chemical inputs to microbially-mediated nutrient management addresses significant environmental issues associated with conventional agriculture (Atapattu *et al*., 2025). Replacing synthetic fertilizers with biofertilizers reduces production costs, maintains soil fertility, prevents water resource contamination, and promotes long-term agricultural sustainability (Srivastav *et al*., 2024; Asoegwu *et al*., 2020). This approach aligns with the principles of ecological intensification, where biological processes replace synthetic inputs without compromising productivity, representing a significant advancement in sustainable agricultural methodology.

The embryological structure of *N. sativa* seeds provides additional insight into the observed responses. With a large proportion of seed phosphorus localized within the embryonic tissues (Colombo *et al*., 2020), increased phosphorus availability in the rhizosphere directly influences seedling vigor through enhanced embryonic allocation. Under phosphorus-limited conditions, maternal plants prioritize seed longevity over vigor through differential phosphorus partitioning to the seed coat (Seyedi *et al*., 2016), which explains the significant correlation between phosphorus availability and seedling vigor establishment observed in our study.

4. Conclusion

This study demonstrates that the integrated application of *A. chroococcum* micronutrient seed coating + biofertilizer substrate significantly enhances the establishment of black cumin seedlings. This improvement is reflected in growth parameters, including a SEP of 80% and an SVI of 6.68, along with reduced oxidative damage, as evidenced by lower levels of H_2O_2 (0.2 μmol g^{-1} FW) and MDA (15.24 nmol g^{-1} FW), due to the activation of the antioxidant defense system. While this biological approach consistently outperformed chemical fertilization in terms of growth and stress mitigation, chemical fertilization produced the highest total antioxidant capacity (86.8%) and phenolic content (87.4 mg GAE g^{-1} FW). This indicates a potential trade-off between growth vigor and secondary metabolite accumulation, which warrants further investigation. These findings support the development of sustainable cultivation protocols that optimize both productivity and phytochemical quality in medicinal plants.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All co-authors have thoroughly reviewed the final version and officially confirm their approval for submission.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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