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Original Article

Cold Plasma Seed Priming Alleviates Salinity Stress in Black Cumin (*Nigella sativa* L.) by Enhancing Phenolic Content, Essential Oil Biosynthesis, and *GPPS* Gene Expression

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ABSTRACT

High salt levels in the growing environment can restrict plant development and reduce the accumulation of secondary metabolites in medicinal species. Although cold plasma (CP) seed priming has been reported to improve plant stress tolerance, its effects on essential oil (EO) biosynthesis and geranyl diphosphate synthase (*GPPS*) gene expression under salinity stress remain poorly understood in black cumin (*Nigella sativa* L.). Therefore, this study investigated the effects of CP seed priming (0, 50, and 100 s) on growth, oxidative status, phenolic compounds, EO production, and *GPPS* expression in black cumin grown under 0, 60, and 120 mM NaCl salinity. Salinity significantly affected plant growth and biochemical traits. Severe salinity (120 mM NaCl) reduced shoot and root dry weight by 40% and 34%, respectively, while increasing H_2O_2 accumulation by 216% compared with the control. In contrast, moderate salinity (60 mM NaCl) enhanced secondary metabolism, increasing EO content by 51%, total flavonoid content by 33%, and *GPPS* expression by 276%. CP priming reduced salt-induced oxidative damage and improved plant performance. The combination of 60 mM salinity and CP treatment for 100 s resulted in the highest total phenolic content, EO content, and *GPPS* expression, increasing these traits by 37%, 81%, and 298%, respectively, relative to the control. Principal component analysis revealed strong positive associations among *GPPS* expression, EO traits, and phenolic compounds. Overall, CP seed priming enhanced salinity tolerance and stimulated terpenoid biosynthesis, highlighting its potential as a sustainable strategy to improve the productivity and phytochemical quality of black cumin under saline conditions.

Keywords: Essential oils, Non-thermal plasma, *GPPS* expression, Saline soil, Secondary metabolites

INTRODUCTION

Salt stress severely limits global crop productivity. Excess salt in soil disrupts plant growth by causing osmotic stress, ion toxicity, and oxidative damage. These changes weaken photosynthesis, limit biomass production, and reduce crop yield and quality [1]. To cope with salinity-induced oxidative stress, plants activate a range of defense mechanisms, including the production of antioxidant compounds such as phenolics and flavonoids, which help maintain cellular redox homeostasis [2, 3].

Nigella sativa L., commonly known as black cumin, is widely cultivated and used for its medicinal, nutritional, and industrial benefits. The seeds of black cumin are rich in bioactive compounds and essential oils (EOs), particularly monoterpenes, which contribute to their therapeutic properties [4]. However, salinity stress can negatively affect the growth, yield, and secondary metabolite production of black cumin. Therefore, the development of sustainable and environmentally friendly approaches to enhance salinity tolerance and maintain EO production in this species is of considerable interest [5].

Cold plasma (CP) seed priming has recently been recognized as a sustainable non-chemical technique to enhance seed vigor and strengthen plant resistance to stress conditions. CP produces reactive oxygen and nitrogen species that act as signaling molecules, stimulating physiological and biochemical processes associated with germination, growth, and stress adaptation [5]. Earlier research has shown that CP treatment can increase antioxidant activity, nutrient uptake, photosynthetic efficiency, and secondary metabolite accumulation under various environmental stresses [6-8]. Furthermore, CP can regulate stress-responsive genes, thereby improving plant resilience to adverse conditions [9].

EO biosynthesis in black cumin is closely associated with the terpenoid pathway, in which geranyl diphosphate synthase (*GPPS*) plays a key regulatory role. *GPPS* catalyzes the formation of geranyl diphosphate, a fundamental precursor for monoterpene synthesis and EO production [10,11]. Changes in *GPPS* expression may therefore directly influence the accumulation of EO constituents under stress conditions and following priming treatments. Moreover, stress-induced changes in phenolic content, driven by the phenylpropanoid pathway, may complement terpenoid-based defense mechanisms, reflecting coordinated regulation of secondary metabolism in response to environmental cues [12].

Given the limited information regarding the effects of cold plasma on EO biosynthesis and *GPPS* gene expression in black cumin under salinity stress, the present study aimed to investigate the potential of CP seed priming to alleviate salinity-induced damage. Specifically, we evaluated its effects on plant growth, phenolic content, EO yield, antioxidant responses, and *GPPS* gene expression in *N. sativa* subjected to different salinity levels. We hypothesized that CP seed priming would enhance salinity tolerance by stimulating antioxidant defenses and terpenoid biosynthesis, thereby improving plant performance and EO production under saline conditions.

MATERIALS AND METHODS

Growth Conditions

In 2024, the experiment was conducted in a greenhouse using a factorial arrangement in a completely randomized design (CRD) with three replicates. The experiment included two factors: salinity stress at three NaCl concentrations (0, 60, and 120 mM) and cold plasma (CP) seed priming at three levels: control, CP treatment for 50 s, and CP treatment for 100 s.

The treatment levels were selected based on previous studies and preliminary germination tests conducted on black cumin (*Nigella sativa* L.). Prior to sowing, seeds were surface-sterilized and planted in 3-L plastic pots filled with loamy soil (pH 7.1, EC 1.1 dS m^{-1}) in April 2024. Greenhouse conditions were maintained at mean day/night temperatures of 27 ± 2 °C and 17 ± 2 °C, respectively, at a relative humidity range of 60–70%. Six seeds were sown in each pot, and after seedling establishment, the plants were thinned to three uniform seedlings per pot.

Cold plasma treatment was applied to the seeds for either 50 or 100 s before sowing, according to the experimental design. Salinity stress was imposed at the six-leaf stage through irrigation with saline water containing the designated NaCl concentrations for 40 days.

A total of nine treatment combinations (3 salinity levels × 3 seed-priming levels) were evaluated, resulting in 27 experimental units (pots). Samples for determination of plant biomass, hydrogen peroxide (H_2O_2) content, total phenolic content (TPC), and total flavonoid content (TFC), EO percentage and yield, and *GPPS* gene expression were collected at physiological maturity (90 days after sowing; BBCH 79). Seed yield components and seed-related traits were assessed at full maturity (110 days after sowing; BBCH 99). The total growth period lasted 110 days.

Cold Plasma Characteristics

CP treatment was performed using an atmospheric-pressure dielectric barrier discharge (DBD) system. Plasma was generated at an applied voltage of 11 kV and a frequency of 23 kHz using high-purity argon as the working gas at a flow rate of 2 L min^{-1}. The discharge was produced between two glass-covered copper electrodes separated by an electrode gap of 3 mm. The plasma was operated under non-thermal conditions, with the gas temperature maintained at approximately 30 °C to avoid thermal damage to the seeds [13].

Plant Weight

Shoot and root dry weights were determined by harvesting the plants and separating the shoots and roots. The samples were shade-dried under ambient laboratory conditions (25 ± 2 °C) in a well-ventilated room until a constant weight was reached, which was defined as two consecutive weight measurements taken at 24-h intervals differing by less than 1%. Shade-drying was used to minimize the loss of volatile compounds and preserve heat-sensitive metabolites. The dried samples were then weighed using a digital balance [14].

Total Phenolic Content

The TPC was estimated using the Folin–Ciocalteu colorimetric procedure. In this assay, a measured volume of plant extract was combined with Folin–Ciocalteu reagent and sodium carbonate solution to initiate the color reaction. Following dark incubation, absorbance was read at 765 nm. Quantification was performed against a gallic acid standard curve, and the data were presented as mg GAE g^{-1} DW [15].

Total Flavonoid Content

The flavonoid content of the samples was estimated through an $AlCl_3$-based colorimetric assay. Briefly, the extract was treated with aluminum chloride solution and kept at ambient temperature to complete the reaction. Absorbance was recorded at 415 nm using a spectrophotometer. A quercetin calibration curve was applied for quantification, and the results were reported as mg QE g^{-1} DW [16].

Essential Oils

EO was determined from air-dried seeds using hydrodistillation with a Clevenger-type apparatus for 3 h. A known weight of dried seeds was subjected to distillation, and the obtained EO was separated from water, collected, and measured volumetrically. EO content was expressed as % (v/w)**,** representing mL of EO per 100 g of seed dry weight. EO yield was calculated by multiplying the oil content by the corresponding dry seeds and expressed as mL per plant [17].

Hydrogen Peroxide Content

H_2O_2 was determined according to a spectrophotometric method. Fresh leaf samples were homogenized in cold trichloroacetic acid (TCA) and centrifuged to obtain the supernatant. The reaction mixture was prepared by adding potassium phosphate buffer and potassium iodide (KI) to the extract. Following dark incubation, the reaction mixture was analyzed spectrophotometrically at 390 nm. Hydrogen peroxide content was quantified from a standard calibration curve and reported as μmol g^{-1} FW [18].

Geranyl Diphosphate Synthase Expression

Total RNA was extracted from leaf samples using a commercial RNA extraction kit (Denazist, Iran) according to the manufacturer's instructions. Approximately 50 mg of leaf tissue was ground in liquid nitrogen, and RNA was purified using chloroform extraction followed

by silica column purification. RNA quality and concentration were evaluated using a NanoDrop spectrophotometer (Thermo Scientific 2000c, USA) and confirmed by 1% agarose gel electrophoresis. Residual genomic DNA was removed by DNase I treatment, and first-strand cDNA was synthesized from 500 ng of total RNA using oligo(dT) and random primers with reverse transcriptase (SMOBio, Taiwan). Quantitative real-time PCR (qPCR) was performed on a LightCycler system (Roche, Switzerland) using SYBR Green chemistry. Each 20-µL reaction contained 10 µL of 2× SYBR Green Master Mix, 0.5 µM of each forward and reverse primer, 2 µL of cDNA template, and nuclease-free water. The amplification program consisted of an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. A melting curve analysis (65–95 °C) was performed after amplification to verify the specificity of the PCR products. Primer sequences are listed in Table 1. The 18S rRNA gene was used as the internal reference gene because of its stable expression under the experimental conditions. Each biological sample was analyzed with three technical replicates. Three independent biological replicates were analyzed for each treatment, and each biological replicate was measured in three technical replicates. Relative *GPPS* expression was calculated using the 2^−ΔΔCt method [19]. Primer specificity was confirmed by a single peak in the melting curve, and primer amplification efficiencies were within the acceptable range of 90–110% with correlation coefficients (R^2) greater than 0.99.

Table 1 Primer sequences and characteristics used for *GPPS* gene expression analysis in *Nigella sativa*.

Gene	Primer sequence (5'–3')	Tm (°C)	Product size (bp)
GPPS (F)	GAAGCCAAACAGACCGGAAC	59.4	134
GPPS (R)	ATTATACGACGGGCGGTACC	59.6	134
18S (F)	GGAGTATGGTCGCAAGGCTGAAAC	59.3	133
18S (R)	CTCAGTCTGTCAATCCTTACTATGTCTGG	59.2	133

Statistical Analysis

The collected data were analyzed statistically using SAS software. Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was examined using Levene's test. The collected data were then analyzed statistically using SAS software. Treatment means wereTreatment means were compared with Duncan's multiple range test at the 5% significance level. Principal component analysis (PCA) was conducted using XLSTAT software (version 2019.2.2.59614; Addinsoft, Paris, France) implemented in Microsoft Excel (Microsoft Office 16.0).

Results

Plant Weight

Shoot weight was not significantly affected by CP treatment under non-saline conditions (Table 2), remaining close to the control value (2.83 g). However, salinity reduced shoot weight by approximately 11.7% and 40% at 60 and 120 mM NaCl, respectively, in untreated plants. Under 120 mM salinity, shoot weight remained reduced by 30% and 28% in CP 50 s- and CP 100 s-treated plants, respectively, compared with the non-saline control, whereas the untreated plants exhibited a 40% reduction (Fig. 1a).

Salinity stress significantly reduced root weight, with decreases of 14% and 34% at 60 and 120 mM NaCl, respectively, in untreated plants. Cold plasma treatment alleviated these reductions, as root weight under 60 mM salinity was only 4% lower than the control in both CP 50 s- and CP 100 s-treated plants. Under 120 mM, CP 50 s and CP 100 s treatments limited the reduction in root weight to 16% relative to the control, whereas untreated plants exhibited a 34% decline (Fig. 1 b).

Table 2 Analysis of variance for the effects of salinity and cold plasma on physiological and biochemical traits of black cumin.

Source of variation	df	MS Shoot weight	Root weight	TPC	TFC	EO	EO yield	H_2O_2	*GPPS*
Salinity (S)	2	0.404 **	0.404 **	53.861 **	13.523 **	0.0743 ***	14.008 **	47.974 **	17.074 **
Cold plasma (CP)	2	0.101 **	0.101 **	7.930 **	0.297 ns	0.0145 **	8.224 **	2.801 **	0.165 **
S × CP	4	0.012 *	0.0122 *	1.396 ***	0.711 *	0.0003 *	0.332 *	0.738 **	0.013 *
Error	18	0.0038	0.0038	0.1319	0.2132	0.0001	0.1382	0.1153	0.0041
CV (%)	–	4.02	4.02	1.69	6.45	3.23	7.2	7.88	6.57

Values are mean squares (MS). ns = not significant; * and ** indicate significance at $P \leq 0.05$ and $P \leq 0.01$, respectively. CV = coefficient of variation.

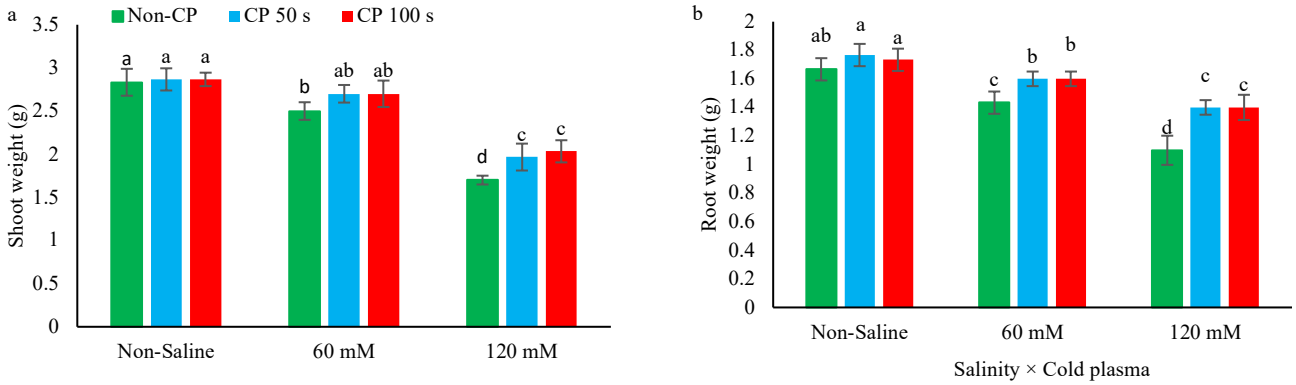

Fig. 1 Effects of salinity stress and cold plasma (CP) seed priming on shoot weight (a) and root weight (b) of black cumin plants. Data are presented as the mean ± standard error (SE) of three biological replicates (n = 3). Different lowercase letters indicate significant differences among treatment means according to Duncan's multiple range test at $P \leq 0.05$.

Phenolic and Flavonoid Content

TPC was significantly influenced by both CP treatment and salinity stress, indicating a strong interaction between abiotic stress and pre-sowing seed stimulation (Table 2). Overall, salinity and CP treatments acted synergistically to enhance the accumulation of phenolic compounds. The highest total TPC was recorded under 60 mM salinity combined with CP treatments of 50 and 100 s, which resulted in increases of 36% and 37%, respectively, relative to the control (Fig. 2 a).

TFC also showed a pronounced response to salinity even in the absence of CP treatment. Under non-CP conditions, salinity levels of 60 and 120 mM increased TFC by 33% and 22%, respectively, compared with the control, indicating that salinity alone is a key driver of flavonoid accumulation (Fig. 2 b). In addition, under non-saline conditions, CP treatments of 50 and 100 s led to modest but significant increases in TFC of 10% and 12%, respectively, relative to the control, demonstrating that CP can independently enhance phenolic biosynthesis even without salt stress.

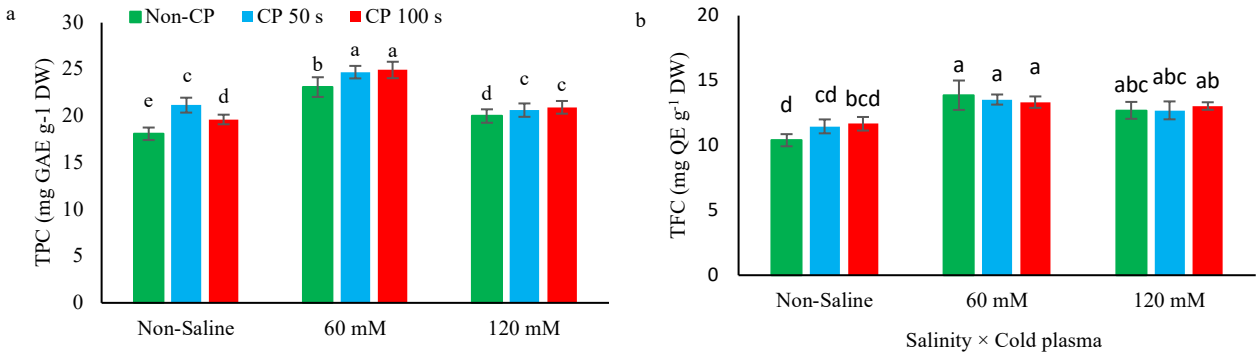

Fig. 2 Effects of salinity stress and cold plasma (CP) seed priming on total phenolic content (TPC, a) and total flavonoid content (TFC, b) of black cumin plants. Data are presented as the mean ± standard error (SE) of three biological replicates (n = 3). Different lowercase letters indicate significant differences among treatment means according to Duncan's multiple range test at $P \leq 0.05$.

Essential Oils

EO content was significantly enhanced by both moderate salinity and CP priming. The highest EO percentage was recorded under 60 mM salinity combined with CP treatment for 100 s, resulting in an 81% increase relative to the control. Under non-CP conditions, salinity at 60 and 120 mM increased EO content by 51% and 18%, respectively, compared with non-saline plants, indicating that moderate salinity is more effective than severe salinity in stimulating EO biosynthesis. In addition, at 120 mM salinity, CP treatments of 50 and 100 s further increased EO content by 13% and 22%, respectively, relative to non-CP-treated plants (Fig. 3a), demonstrating the enhancing role of CP under high salinity conditions.

A similar trend was observed for EO yield, although with some differences due to biomass-related effects. EO yield increased by 17% under 60 mM salinity in non-primed plants, while it decreased by 28% under 120 mM salinity without CP treatment, indicating that severe salinity negatively affects overall productivity despite its influence on EO concentration. Importantly, the maximum EO yield was achieved under the combined treatment of 60 mM salinity and CP exposure for 100 s, which resulted in a 76% increase relative to the control (Fig. 3 b).

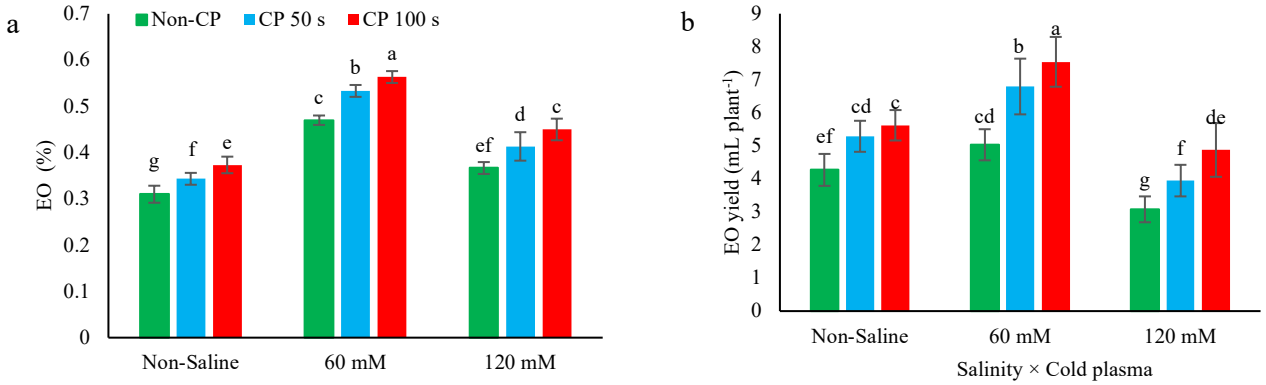

Fig. 3 Effects of salinity stress and cold plasma (CP) seed priming on essential oil percentage (EO, a) and EO yield (b) of black cumin plants. Data are presented as the mean ± standard error (SE) of three biological replicates (n = 3). Different lowercase letters indicate significant differences among treatment means according to Duncan's multiple range test at $P \leq 0.05$.

Hydrogen Peroxide and Geranyl Diphosphate Synthase Gene Expression

H_2O_2 accumulation was significantly influenced by both salinity stress and CP treatment, reflecting changes in the plant's oxidative status. Relative to the control treatment, H_2O_2 content increased progressively with increasing salinity, indicating enhanced oxidative stress under saline conditions. At 60 mM salinity, H_2O_2 increased by 61% in the absence of CP, while at 120 mM salinity it increased by 215%, representing the highest value recorded. Application of CP reduced H_2O_2 accumulation under both salinity levels, with the greatest reduction observed under 120 mM salinity, where CP for 100 s lowered the increase from 215% to 132% relative to the control (Fig. 4 a).

Relative to the control treatment, *GPPS* expression was significantly upregulated by both salinity and CP. Under non-saline conditions, CP treatment slightly increased *GPPS* expression by 23% and 22% following 50 and 100 s exposure, respectively, compared with the control. Salinity alone strongly induced *GPPS* transcription, with expression levels increasing by 275% at 60 mM and 86% at 120 mM salinity. The greatest *GPPS* expression was observed at 60 mM salinity, suggesting that moderate salt stress is a potent inducer of terpenoid biosynthesis. Moreover, CP treatment further enhanced *GPPS* expression under saline conditions. At 60 mM salinity, *GPPS* expression increased by 281% and 298% under CP treatments of 50 and 100 s, respectively, relative to the control. Similarly, at 120 mM salinity, *GPPS* expression was elevated by 114% and 120 % under CP 50 and 100 s, respectively (Fig. 4b).

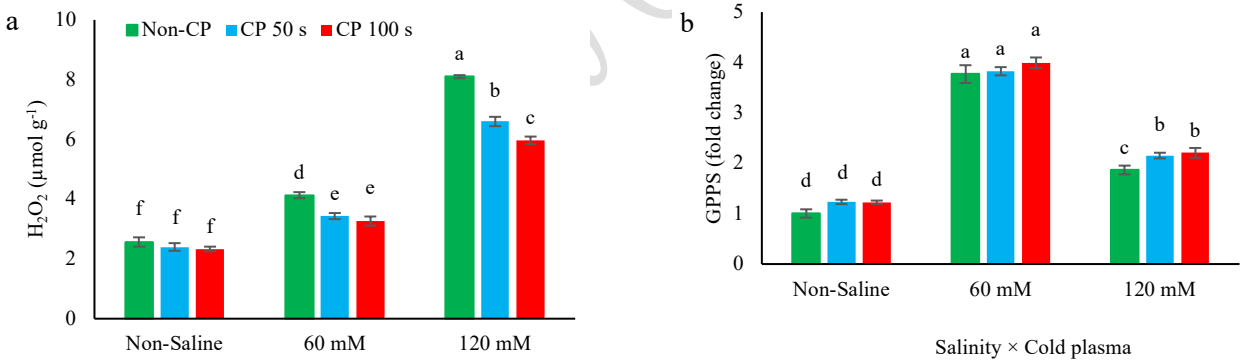

Fig. 4 H_2O_2 (a) and geranyl diphosphate synthase (*GPPS*) expression (b) of black cumin plants under salinity stress and seed priming with cold plasma (CP). Data are presented as the mean ± standard error (SE) of three biological replicates (n = 3). Different lowercase letters indicate significant differences among treatment means according to Duncan's multiple range test at $P \leq 0.05$.

Principal Component Analysis

PCA was performed to evaluate relationships among physiological, biochemical, and gene expression traits under different salinity treatments. The first two principal components (PC1 and PC2) explained 100% of the total variance, with PC1 accounting for 55% and PC2 for 45%. PC1 was mainly associated with *GPPS* expression, EO, EO yield, TPC, and TFC, indicating a strong positive relationship among secondary metabolite-related traits. In contrast, PC2 was strongly influenced by H_2O_2, shoot dry weight, and root dry weight, reflecting variation in oxidative stress and biomass accumulation. Score analysis showed clear separation among treatments. The 60 mM NaCl treatment was positively associated with PC1, indicating higher levels of *GPPS*, EO, EOY, TPC, and TFC, whereas the control treatment was also positively related to PC2. In contrast, the 120 mM NaCl treatment was strongly associated with negative PC2 values, indicating higher stress conditions characterized by increased H_2O_2 accumulation and reduced biomass (Fig. 5 a).

PCA of the CP treatments showed that PC1 and PC2 explained 96.3% and 3.7% of the total variation, respectively. PC1 was strongly associated with all measured traits, including *GPPS* expression, EO content and yield, H_2O_2, shoot and root dry weight, total phenolic content, and total flavonoid content, indicating that it represented the overall variation in physiological and biochemical responses. The score plot clearly

separated the control from the cold plasma-treated samples (50 and 100 s), with the greatest separation observed for the 100 s treatment. Although the cold plasma treatments were positioned on the negative side of PC1, the direction of the principal component axis is arbitrary and does not indicate reduced plant performance. Rather, the separation along PC1 reflects the distinct physiological and biochemical responses induced by CP seed priming relative to the untreated control (Fig. 5b).

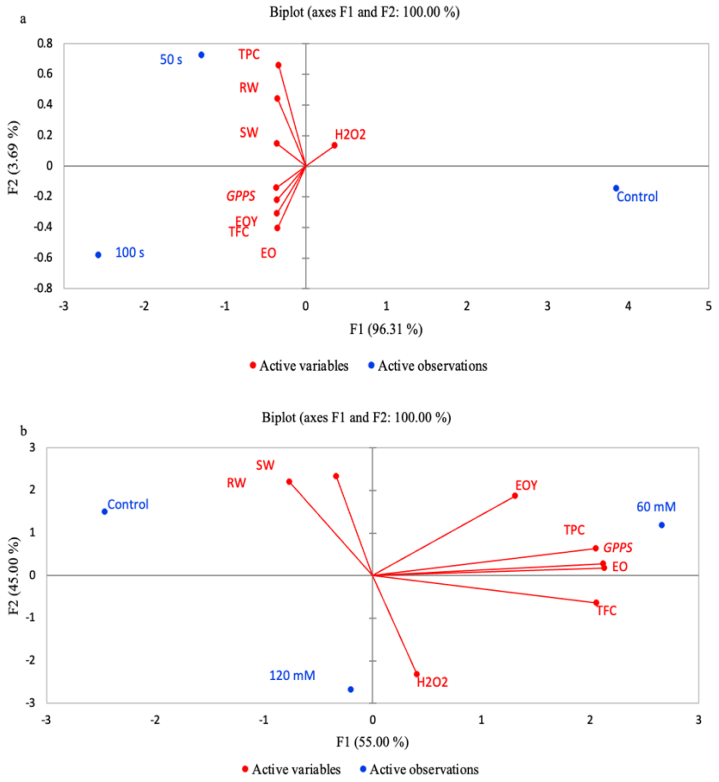

Fig. 5 PCA biplots of the evaluated traits in response to salinity levels (a) and cold plasma (CP) seed priming (b).

DISCUSSION

Salinity stress generally decreases plant weight because it creates both osmotic stress and ionic toxicity. Excess salts in the root zone restrict water absorption, creating drought-like conditions even when soil water is available. This results in reduced cell expansion, slower growth, and lower photosynthetic activity. In addition, excess sodium (Na^+) and chloride (Cl^-) ions disrupt enzyme function, damage chloroplasts, and interfere with nutrient uptake such as potassium and calcium, which are essential for growth. Together, these effects reduce biomass accumulation, so both root and shoot fresh and dry weights decline [20-22]. CP seed priming improves plant weight under salinity stress by enhancing early seed vigor and strengthening stress tolerance mechanisms. CP treatment can increase seed surface permeability, improve water uptake during germination, and activate metabolic and hormonal pathways that promote faster and more uniform germination [23]. More importantly, it stimulates antioxidant defense systems, helping plants reduce oxidative damage caused by salt-induced ROS [20]. It can also enhance photosynthetic efficiency and secondary metabolism, leading to better growth and biomass production even under stressful conditions. In black cumin, these improvements translate into higher root and shoot development, ultimately increasing plant weight compared to untreated stressed plants. The increase in shoot weight of sage plants under salinity stress following CP seed priming was also reported by Alawamleh et al. [7], which supports our findings regarding the positive effect of CP treatment on plant biomass accumulation.

Salinity stress markedly influenced the accumulation of phenolic and flavonoid compounds in black cumin, with salinity at 60 mM promoting their biosynthesis. The increase in TPC and TFC at 60 mM salinity is likely associated with the activation of defense mechanisms in response to moderate stress. Salinity-induced oxidative stress stimulates the production of ROS, which serve as signals for the synthesis of antioxidant secondary metabolites, including phenolics and flavonoids [1,24]. These compounds help protect cellular structures by scavenging free radicals and reducing oxidative damage. However, at 120 mM salinity, although TPC and TFC remained elevated compared with the control, a slight decline was observed. This reduction may be attributed to the severity of the stress, which can impair photosynthesis, limit carbon assimilation, and reduce the availability of metabolic precursors required for secondary metabolite biosynthesis. Under excessive salinity, cellular damage may also exceed the plant's capacity to maintain high levels of phenolic and flavonoid production [6]. CP seed priming further enhanced TPC and TFC under both normal and saline conditions by stimulating physiological and biochemical processes related to stress tolerance. CP-generated reactive species act as signaling molecules that activate genes and enzymes involved in the phenylpropanoid pathway, leading to increased synthesis of phenolic and flavonoid compounds. Moreover, CP treatment can improve seed vigor, nutrient uptake, photosynthetic performance, and antioxidant enzyme activity, enabling plants to maintain a stronger metabolic status under stress [6,7]. As a result, CP-primed plants accumulated greater amounts of TPC and TFC than non-primed plants, reflecting an enhanced antioxidant defense system and improved

adaptation to salinity stress. The findings of the current study are supported by Shirkhani and Esmaeili [1], who demonstrated that cold plasma treatment under moderate salinity conditions led to the greatest accumulation of TPC and TFC in *Carthamus tinctorius*.

EO content and yield rose at 60 mM salinity, probably due to mild stress-induced stimulation of secondary metabolite accumulation. Moderate stress may also promote the allocation of carbon resources toward secondary metabolite production rather than primary growth processes [25]. However, at 120 mM salinity, EO yield showed a declining trend, likely due to the detrimental effects of severe salt stress on plant physiology. Excessive salinity can impair photosynthesis, reduce nutrient uptake, disrupt enzymatic activities, and limit the availability of metabolic precursors required for EO biosynthesis, thereby decreasing EO accumulation [7]. CP seed priming further enhanced EO yield by improving plant growth, metabolic activity, and stress tolerance. CP treatment can stimulate enzymatic processes and activate signaling pathways associated with the biosynthesis of terpenoids, the major constituents of EO [26]. In addition, CP improves water uptake, nutrient acquisition, and antioxidant capacity, enabling plants to maintain higher photosynthetic efficiency and metabolic performance under both normal and saline conditions. These improvements increase the availability of energy and carbon skeletons necessary for EO production, resulting in greater EO production in CP-primed plants compared with untreated plants [7,26].

Salinity stress increased H_2O_2 accumulation as a consequence of enhanced oxidative stress in plant cells. High salt concentrations disrupt cellular homeostasis and impair normal metabolic processes, particularly photosynthesis and respiration, leading to excessive production of ROS, including H_2O_2 [27]. Although H_2O_2 functions as an important signaling molecule at low concentrations, its excessive accumulation under salinity stress can cause oxidative damage to lipids, proteins, and nucleic acids. Elevated H_2O_2 levels under saline conditions reflect an imbalance between reactive oxygen species (ROS) generation and the plant's antioxidant defense capacity. In contrast, CP seed priming reduced H_2O_2 accumulation by enhancing the plant's antioxidant defense system and improving its ability to cope with salinity-induced oxidative stress [28,29]. CP treatment can stimulate the activity of antioxidant enzymes, which efficiently detoxify ROS and prevent their excessive buildup. Furthermore, CP promotes the accumulation of non-enzymatic antioxidants, including phenolic and flavonoid compounds, which contribute to ROS scavenging. As a result, CP-primed plants maintain better redox homeostasis and exhibit lower H_2O_2 levels than non-primed plants under salinity stress, indicating reduced oxidative damage and enhanced stress tolerance [8,30].

The *GPPS* gene is a key regulatory gene involved in EO biosynthesis in black cumin, as it provides the precursor molecules required for monoterpene production [31]. The expression of *GPPS* increased under 60 mM salinity, likely as part of an adaptive response aimed at enhancing terpenoid and EO biosynthesis. Moderate salt stress can act as an elicitor that stimulates secondary metabolic pathways and activates stress-responsive genes involved in the synthesis of protective compounds [32]. Since *GPPS* is a key enzyme in the terpenoid biosynthetic pathway and provides precursors for EO production, its upregulation under 60 mM salinity may contribute to the increased accumulation of EOs observed under moderate stress conditions. In contrast, although *GPPS* expression remained higher than in the control under 120 mM salinity, it declined relative to 60 mM. This reduction likely reflects a trade-off between primary and secondary metabolism under severe stress, whereby plants preferentially allocate available energy and carbon resources to primary metabolism and essential cellular maintenance rather than to the synthesis of secondary metabolites. Moreover, severe salinity impairs photosynthesis, reduces ATP production and carbon assimilation, and disrupts transcriptional and enzymatic processes, thereby limiting the resources required to sustain high *GPPS* expression and terpenoid biosynthesis [33]. CP seed priming enhanced *GPPS* gene expression under both normal and saline conditions. This effect may be attributed to CP-induced signaling processes that activate stress-responsive and secondary metabolism-related pathways. Furthermore, CP treatment improves antioxidant status and overall physiological performance, creating favorable conditions for gene activation and metabolite biosynthesis. The upregulation of *GPPS* in CP-treated plants suggests stimulation of the terpenoid biosynthetic pathway, which may contribute to increased EO production and improved adaptation to salinity stress [34].

CONCLUSIONS

In conclusion, the results demonstrate that cold plasma seed priming effectively enhances the tolerance of black cumin to salinity stress by stimulating antioxidant defenses and secondary metabolite biosynthesis. While salinity-imposed growth limitations and increased oxidative stress, moderate salinity acted as an elicitor that promoted the accumulation of phenolic compounds and essential oils. Cold plasma priming further strengthened these responses, reducing hydrogen peroxide accumulation and improving plant performance under both moderate and severe salinity conditions. These findings indicate that cold plasma-induced physiological and biochemical adjustments contribute to a more efficient stress adaptation strategy in black cumin. The enhanced accumulation of phenolic compounds and essential oils in cold plasma-primed plants suggests that cold plasma promotes the activation of key metabolic pathways involved in plant defense and product quality. In particular, the upregulation of *GPPS* expression under cold plasma treatment, especially under moderate salinity, highlights the importance of terpenoid pathway activation in the observed increase in essential oil production. The close association among *GPPS* expression, essential oil traits, and phenolic compounds further suggests coordinated regulation of secondary metabolism in response to cold plasma priming. A limitation of the present study is that the chemical composition of the essential oil was not analyzed; therefore, it was not possible to determine how cold plasma and salinity affected the relative abundance of individual essential oil constituents. Therefore, cold plasma seed priming represents a promising, environmentally friendly approach for improving salinity tolerance while simultaneously enhancing the phytochemical and economic value of black cumin. Future studies should characterize essential oil composition and investigate the long-term effects of cold plasma treatment on individual terpenoid constituents and the regulation of other genes involved in terpenoid and phenylpropanoid biosynthesis under different environmental conditions.

Data Availability

The data are available upon request.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Not Applicable.

Authors' Contributions

All authors contributed to drafting, reviewing, and editing the manuscript.

Acknowledgments

Not Applicable.

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