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Physiological and Biochemical Responses of *Pimpinella anisum* to Salinity Stress

Esmail Nabizadeh[1]*, Khadijeh Ahmadi[2] and Soraya Attaee[3]

[1] *Institute of Agriculture, Water, Food, and Nutraceuticals, Mah.C., Islamic Azad University, Mahabad Branch, Mahabad, Iran*
[2] *Faculty of Agricultural Sciences, Shahed University, Tehran, Iran*
[3] *Marand Branch, Islamic Azad University, Marand, Tabriz, Iran*

*Corresponding author: Email: nabizadeh.esmaeil@gmail.com

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ABSTRACT

Salinity stress represents a significant abiotic constraint that adversely affects growth, physiological performance, and secondary metabolite production in medicinal and aromatic plants. This study evaluated the impact of varying salinity levels (0, 30, 60, 90, 120, and 150 mM NaCl) on the physiological traits and yield components of *Pimpinella anisum* L. under greenhouse conditions using a completely randomized design. Salinity significantly influenced all measured parameters. Increasing NaCl concentrations induced a progressive decline in chlorophyll a and b contents, while relative water content (RWC) remained relatively stable up to 120 mM before dropping sharply at 150 mM. Proline accumulation exhibited a threshold-dependent response, decreasing at 30 mM but increasing significantly from 60 mM onward. Notably, plants subjected to 120 and 150 mM NaCl failed to reach harvest stage due to severe stress-induced mortality. Essential oil content in both seeds and leaves peaked at 60 mM NaCl, indicating that moderate salinity stimulates secondary metabolite biosynthesis, whereas 90 mM NaCl caused a marked decline, particularly in seeds. These findings highlight the sensitivity of *P. anisum* to high salinity and suggest that controlled, moderate saline conditions may enhance phytochemical yield, underscoring the need for optimized irrigation management in anise cultivation under saline environments.

Keywords: Salinity Stress, *Pimpinella anisum*, Essential Oil, Photosynthetic Pigments, Proline

INTRODUCTION

In modern horticultural and medicinal crop production, environmental stresses, particularly salinity, pose significant challenges to sustainable agriculture. Salinity stress adversely impacts plant growth and metabolic functioning by inducing osmotic stress, leading to reduced water uptake, and by causing ion toxicity, primarily due to excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions in plant tissues. These ionic imbalances disrupt essential physiological and biochemical processes, including nutrient acquisition, enzyme activity, and hormonal regulation, ultimately compromising plant productivity and quality [1, 2]. Medicinal and aromatic plants are especially vulnerable to environmental fluctuations, necessitating precise agronomic and physiological management, particularly under abiotic stress conditions. These species are cultivated primarily for their bioactive secondary metabolites, such as alkaloids, phenolic compounds, terpenoids, and essential oils, which have widespread applications in pharmaceutical, nutraceutical, food, and cosmetic industries [3]. The quantity and composition of these metabolites are highly responsive to environmental cues, including salinity, which can either inhibit or stimulate their biosynthesis depending on stress severity and plant genotype.

Pimpinella anisum L. (anise) is a highly valued medicinal and aromatic plant cultivated for its seeds, which are rich in anethole and estragole, two major phenylpropanoid compounds that constitute its essential oil. These constituents exhibit a broad spectrum of therapeutic properties, including antimicrobial, antifungal, antiviral, antioxidant, anti-inflammatory, anticancer, and antigenotoxic effects [3, 4]. However, the biosynthetic pathways responsible for the production of such secondary metabolites are intricately regulated by environmental factors. Salinity stress, in particular, can reduce photosynthetic efficiency, alter carbon and nitrogen metabolism, and disrupt the availability of metabolic precursors required for secondary metabolite synthesis [5]. Interestingly, mild to moderate salinity stress may act as a metabolic elicitor, triggering the plant's defense mechanisms and enhancing the accumulation of secondary metabolites such as terpenoids, flavonoids, and antioxidants [6]. This phenomenon is attributed to the activation of stress-responsive signaling pathways and transcriptional regulation of key biosynthetic enzymes. Nonetheless, the effect of salinity on plant performance is highly dependent on the intensity, duration, and timing of exposure, as well as the developmental stage of the plant.

Understanding the salinity tolerance threshold of medicinal plants like *P. anisum* is therefore critical for developing effective and site-specific cultivation strategies in saline-prone environments. The salinity threshold refers to the maximum salt concentration in the soil or irrigation water that a plant can tolerate without significant reductions in growth, yield, or metabolic performance. Identifying this threshold enables growers and researchers to design irrigation regimes, select appropriate soil amendments, and implement stress mitigation practices tailored to a plant's physiological limits [7, 8]. The urgency of this understanding has grown with the increasing prevalence of soil salinization, a phenomenon that now affects over 20% of irrigated lands globally and continues to expand, particularly in arid and semi-arid regions. Several

interrelated factors contribute to this trend, including prolonged droughts and rising evapotranspiration rates associated with climate change, the overexploitation of freshwater resources, the use of saline or marginal-quality water for irrigation, and inadequate drainage systems. In such settings, salts accumulate in the root zone, reducing soil osmotic potential and hindering water and nutrient uptake by plants [3, 4].

For medicinal and aromatic plants, which are often more sensitive to abiotic stresses than staple crops, the consequences of salinity can be especially severe. Reduced growth, impaired physiological function, and diminished secondary metabolite production not only lower yields but also compromise the quality and therapeutic efficacy of plant-derived products. In crops like anise, where the commercial value lies in the biosynthesis of specific essential oil components, even moderate salinity stress can shift metabolic profiles and reduce marketability [9, 10]. Therefore, addressing salinity stress is not only essential for maintaining plant health and productivity but also for safeguarding the economic viability and medicinal quality of anise and similar species. A comprehensive understanding of plant responses to salinity, including tolerance mechanisms such as osmotic adjustment, ion compartmentalization, antioxidant defense activation, and metabolic reprogramming, is fundamental for breeding salt-tolerant cultivars, optimizing agronomic practices, and guiding policy decisions on land and water resource management in vulnerable regions [7, 11].

Salinity affects several physiological parameters, including photosynthetic pigment concentration (chlorophyll a, chlorophyll b, and carotenoids), relative water content (RWC), membrane stability, and osmotic adjustment through the accumulation of compatible solutes such as proline and soluble sugars. These responses collectively determine plant vigor, productivity, and the capacity to maintain secondary metabolite biosynthesis under stress conditions [6]. Accordingly, the present study was undertaken to assess the effects of varying salinity levels on key physiological traits and essential oil yield in *P. anisum* L. under controlled greenhouse conditions. By elucidating the plant's responses to salinity stress, the study aims to inform practical management strategies that can enhance the resilience and productivity of anise in saline environments.

MATERIALS AND METHODS

Experimental Design and Plant Materials

The experiment was carried out during the 2019–2020 growing season in a research greenhouse at the Faculty of Agriculture, Islamic Azad University, Mahabad Branch. A completely randomized design (CRD) was employed with six salinity levels (0, 30, 60, 90, 120, and 150 mM NaCl) and three replications. Seeds of *P. anisum* L. were sourced from Pakan Seed Company, Isfahan, Iran. Following germination tests, seeds with 98% viability and 99% purity were selected for sowing.

Cultivation Conditions and Salinity Treatments

Seeds were sown in 2-kg plastic pots filled with perlite. Each pot measured 35 cm in height and 27.5 cm in top diameter. Ten seeds were initially sown per pot, and after reaching the four-leaf stage, seedlings were thinned to four plants per pot. Pots were irrigated with distilled water for the first four weeks to establish uniform seedling growth. A week after thinning, salinity treatments were initiated by irrigating with Hoagland's nutrient solution supplemented with the designated NaCl concentrations (0, 30, 60, 90, 120, and 150 mM). To prevent localized salt accumulation and maintain homogeneous root-zone salinity, a controlled leaching irrigation with distilled water was applied twice during the experimental period (at the sixth week and one week prior to harvest). This leaching fraction represented less than 10% of the total irrigation volume and was uniformly applied to all pots, including the control, ensuring that target salinity levels remained stable while avoiding toxic salt crust formation near the root surface. Root-zone electrical conductivity (EC) was monitored weekly using a saturated paste extract method to verify treatment consistency throughout the trial, in accordance with standard greenhouse salinity management protocols [12, 13].

Measurement of Physiological Traits

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were determined following pigment extraction in 80% acetone and spectrophotometric measurements at specific wavelengths [14].

1) Chlorophyll a = Chl a = 12.25 × A663 nm − 2.79 × A646 nm
2) Chlorophyll b = Chl b = 21.50 × A646 nm − 5.10 × A663 nm
3) Chlorophyll total = Chl (a+b) = 7.15 × A663 nm + 18.71 × A646 nm
4) Carotenoids = (1000 × A470 nm − 1.82 × Chl a − 85.02 × Chl b)/198

RWC was assessed according to Barrs and Weatherley [15] by measuring fresh weight (FW), turgid weight (TW), and dry weight (DW) of leaf samples.

5) RWC (%)= (FW-DW)/(TW-DW) * 100

The TC content was quantified using the phenol-sulfuric acid method. Absorbance was measured with a spectrophotometer at a wavelength of 485 nm. Standard curves were prepared using solutions with glucose concentrations ranging from 0 to 10 mg/100 mL. Considering the dry weight of the samples, the TC content was calculated as milligrams per gram of sample dry weight [16]. Proline concentration was measured based on the method of Bates *et al.* [17] involving extraction in 3% sulfosalicylic acid, reaction with acid ninhydrin, and absorbance reading at 520 nm. Dry weight (DW) of the plants were quantified to determine Shoot dry weight. At seed maturation, plants were harvested at the base using sterile scissors. Plant material was separated into seeds and leaves, then oven-dried at 40 °C for 72 h to constant weight. Essential oils were extracted separately from dried seeds and dried leaves using a glass Clevenger-type apparatus. For each tissue type, 50 g of dried plant material was hydrodistilled with 500 mL of distilled water for 2 h. The essential oil layer was collected, dehydrated over anhydrous sodium sulfate, and the volume was measured. Results were expressed as µL/g (v/w) based on dry weight. This extraction protocol follows the standardized methodology for *P. anisum* and ensures accurate quantification of tissue-specific essential oil yields [4].

Statistical Analysis

Data were statistically analyzed using SAS software (version 9.1). Mean values were compared using Duncan's multiple range test at a 5% significance level.

RESULTS

The combined analysis of two-year experimental data indicated that salinity stress had a statistically significant effect on leaf pigment traits, including chlorophyll a, chlorophyll b, and carotenoid content. Moreover, the interaction between salinity level and year was also significant at the 1% probability level for several key physiological traits, including relative water content (RWC), carotenoid concentration, and leaf carbohydrate.

Table 1 Mean Comparisons for Salinity Treatments on Various Parameters

Salinity	Rwc (%)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Carotenoids (mg/g FW)	Carbohydrates (µg/g FW)	Proline (µg/g FW)
0 mM	86.15 a	23.37 a	5.25 a	282.89 a	5.31 d	176.83 ab
30 mM	85.81 ab	21.69 ab	4.54 b	243.31 b	8.65d c	147.08 b
60 mM	85.21 bc	20.52 bc	3.98 c	230.2 b	9.71 cd	203.67 a
90 mM	82.58 cd	20.20 bd	3.79 c	212.44 bc	10.65 c	205.25 a
120 mM	81.7 dc	19.19 cd	3.71 c	194.2 c	12.84 ab	209.9 a
150 mM	71.62 f	18.44 d	3.66 c	191.2 c	13.33 a	213.34 a

Means sharing at least one common letter do not differ significantly.

The relative water content (RWC) of *P. anisum* leaves exhibited a salinity-dependent decline, with mean values progressively decreasing as NaCl concentration increased. Under control conditions (0 mM NaCl), RWC reached its maximum value of 86.15% (a), while mild salinity levels (30 and 60 mM) maintained relatively stable water status at 85.81% (ab) and 85.21% (bc), respectively, with overlapping letter groupings indicating minimal differentiation among these treatments. A moderate reduction was observed at 90 mM (82.58%, cd) and 120 mM NaCl (81.70%, dc), where shared letter designations suggest a gradual transitional phase in tissue hydration without abrupt shifts. The most notable change occurred at 150 mM NaCl, where RWC dropped distinctly to 71.62% (f), a mean value bearing no common letter with any other treatment level. This clear separation in letter grouping underscores a marked deviation from the response pattern observed at lower salinities, reflecting a threshold beyond which the plant's capacity to maintain cellular water balance is substantially compromised. Overall, the mean comparison pattern reveals a biphasic trend: relative stability across the 0–120 mM range followed by a pronounced decline at the highest salinity level, highlighting 150 mM NaCl as a critical point affecting water retention in *P. anisum* tissues (Table 2).

Chlorophyll a content in *P. anisum* leaves displayed a progressive decline across increasing salinity levels, with mean values decreasing from 23.37 mg/g (a) under control conditions to 18.44 mg/g (d) at 150 mM NaCl. The mean comparison pattern reveals overlapping letter groupings between adjacent treatments (e.g., ab, bc, bd, cd), indicating a gradual and continuous reduction rather than abrupt shifts. This consistent downward trajectory in mean values suggests that chlorophyll a biosynthesis or stability is increasingly constrained as salinity intensifies, with the most distinct separation observed between the control (a) and the highest salinity level (d).

Chlorophyll b content followed a distinct two-phase response pattern, with mean values dropping from 5.25 mg/g (a) in the control to 4.54 mg/g (b) at 30 mM NaCl, followed by a plateau across 60–150 mM treatments (all sharing the letter c, ranging from 3.98 to 3.66 mg/g). This mean comparison structure indicates that the most pronounced reduction in chlorophyll b occurred at the onset of salinity stress, after which values stabilized at a lower baseline. The clear separation between the control (a), 30 mM (b), and higher salinity levels (c) underscores the heightened sensitivity of chlorophyll b to initial salt exposure, with limited further decline once moderate stress thresholds are exceeded (Table 2).

Carotenoid content in *P. anisum* exhibited a salinity-dependent decline, with mean values decreasing from 282.89 mg/g (a) under control conditions to 191.20 mg/g (c) at 150 mM NaCl. The mean comparison pattern reveals a stepwise reduction: the control treatment (a) maintained the highest carotenoid levels, while mild salinity (30 and 60 mM NaCl) formed an intermediate group (b; 243.31 and 230.20 mg/g, respectively) that was statistically distinct from the control but not from each other. A further decline occurred at moderate to high salinity levels, with 90 mM NaCl (212.44 mg/g, bc) showing partial overlap between the intermediate and lowest groups. The lowest carotenoid means were recorded at 120 and 150 mM NaCl (194.20 and 191.20 mg/g, respectively), both sharing the letter designation (c) and indicating no significant difference between these two highest stress levels. This mean comparison structure underscores a threshold response, where carotenoid content remains relatively stable within each salinity range but drops distinctly between ranges. Overall, the progressive separation of mean values across treatment levels reflects a cumulative impairment of carotenoid biosynthesis or accelerated degradation under increasing salt stress, with the most pronounced reduction occurring beyond 90 mM NaCl (Table 2).

Soluble carbohydrate content in *P. anisum* exhibited a salinity-induced increasing trend, with mean values rising from 5.31 mg/g (d) under control conditions to 13.33 mg/g (a) at 150 mM NaCl. The mean comparison pattern reveals a gradual progression: control (d) maintained the lowest carbohydrate levels, while mild salinity treatments (30 and 60 mM NaCl; 8.65 and 9.71 mg/g, respectively) formed overlapping intermediate groups (dc, cd) that bridged the control and higher stress levels. A moderate increase was observed at 90 mM NaCl (10.65 mg/g, c), which shared partial letter overlap with both lower and higher treatments, indicating a transitional phase in carbohydrate accumulation. The most pronounced elevation occurred at severe salinity levels, where 120 mM NaCl (12.84 mg/g, ab) and 150 mM NaCl (13.33 mg/g, a) formed the highest mean group, with the latter showing complete statistical separation from the control (no shared letters). This mean comparison structure underscores a cumulative osmotic adjustment response, wherein soluble carbohydrates progressively accumulate as salinity

intensifies, likely serving as compatible solutes to maintain cellular turgor and protect metabolic functions. The clear gradient in letter groupings from d (control) to a (150 mM) reflects a dose-dependent enhancement of carbohydrate biosynthesis or reduced utilization under salt stress (Table 2).

Under non-saline control conditions, the proline content was recorded at 176.83 mg/g fresh weight, demonstrating the presence of basal levels of this osmoprotectant in the plant even in the absence of stress. Interestingly, a decrease in proline content was observed under mild salinity (150 mM NaCl), where it dropped to 147.08 mg/g. However, with increasing salinity, the accumulation of proline showed an upward trend: 203.67 mg/g at 60 mM and 205.25 mg/g at 90 mM. This pattern suggests that salinity-induced proline synthesis becomes more pronounced at moderate stress levels (Table 1).

Shoot dry weight in *P. anisum* exhibited a salinity-dependent decline, with mean values decreasing from 1.735 g (a) under control conditions to 0.741 g (c) at 90 mM NaCl. The mean comparison pattern reveals that the 30 mM treatment (1.438 g, ab) shared letter overlap with the control, indicating no significant reduction in biomass accumulation at this mild stress level. A transitional grouping was observed at 60 mM NaCl (0.980 g, bc), which partially overlapped with both the higher and lower treatment groups, reflecting a gradual shift in growth suppression as salinity intensified.

The most pronounced reduction occurred at 90 mM NaCl (0.741 g, c), a mean value that shared no common letter with the control, confirming a statistically distinct decline in shoot biomass under moderate-to-high salinity. Notably, no data were recorded for 120 and 150 mM NaCl treatments, as plants failed to complete their growth cycle under these severe stress conditions. Overall, the mean comparison structure underscores a dose-dependent suppression of vegetative growth, with biomass production remaining relatively stable up to 30 mM NaCl but declining progressively thereafter, highlighting the sensitivity of *P. anisum* shoot development to elevated salinity levels (Table 2).

Table 2 Mean Comparisons for Salinity Treatments on Various Parameters

Salinity	Rwc (%)	Dry Weight of Shoot (gr)	Essential Oil Seed (µL/g)	Essential Oil Leaf (µL/g)
0 mM	86.15 a	1.735 a	11.3 c	4.683 c
30 mM	85.81 ab	1.438 ab	13.288 b	7.288 b
60 mM	85.21 bc	0.980 bc	17.753 a	11.871 a
90 mM	82.58 cd	0.741 c	5.121 d	7.945 b
120 mM	81.7d c	-	-	-
150 mM	71.62 f	-	-	-

Means sharing at least one common letter do not differ significantly.

Seed essential oil content in *P. anisum* exhibited a non-linear, threshold-dependent response to salinity, with mean values ranging from 5.121 to 17.753 µL/g . The mean comparison pattern reveals a progressive enhancement under mild-to-moderate stress: the control treatment (11.30 µL/g) maintained the lowest essential oil accumulation, while 30 mM NaCl (13.288 µL/g) formed a distinct intermediate group. The peak accumulation occurred at 60 mM NaCl (17.753 µL/g), a mean value bearing no common letter with any other treatment, confirming a statistically distinct maximum at this moderate salinity level. A sharp decline was observed at 90 mM NaCl (5.121 µL/g), where the mean value dropped below even the control level and formed a statistically isolated lowest group. This mean comparison structure underscores a biphasic metabolic response: salinity up to 60 mM appears to stimulate secondary metabolite biosynthesis, likely as a protective adaptation, whereas exceeding this threshold triggers a collapse in essential oil production, possibly due to impaired glandular trichome function or resource reallocation toward survival mechanisms. The complete separation of the 90 mM mean from all other treatments highlights this salinity level as a critical turning point for seed metabolic activity (Table 2).

Leaf essential oil content followed a similar but distinct pattern, with mean values increasing from 4.683 µL/g under control conditions to a maximum of 11.871 µL/g at 60 mM NaCl. The mean comparison structure indicates that 30 mM (7.288 µL/g) and 90 mM (7.945 µL/g) treatments shared identical letter groupings, reflecting no significant difference between these two salinity levels despite their numerical disparity. This overlapping designation suggests that leaf essential oil accumulation stabilizes within an intermediate range once salinity exceeds the control condition, with 60 mM representing the optimal induction point. The clear statistical separation between the control, the intermediate plateau, and the peak treatment reveals a more buffered response in leaf tissues compared to seeds. While both organs peaked at 60 mM NaCl, leaves maintained higher essential oil levels at 90 mM than seeds did, indicating potentially greater metabolic resilience or differential regulation of secondary metabolism in vegetative versus reproductive tissues. Overall, the mean comparison gradient supports a model of salinity-induced upregulation of essential oil biosynthesis up to a moderate stress threshold, followed by partial retention rather than complete collapse at higher salinity (Table 2).

DISCUSSION

Salinity stress is considered one of the major environmental constraints limiting plant growth, physiological performance, and secondary metabolite production in medicinal and aromatic plants. The present study demonstrated that increasing salinity levels markedly affected the physiological and biochemical characteristics of *P. anisum*, indicating the sensitivity of this species to saline conditions.

The reduction in relative water content (RWC) under increasing salinity levels indicates the sensitivity of *P. anisum* to osmotic stress. Salt accumulation in the root zone decreases soil water potential and restricts water uptake, leading to reduced cell turgor and impaired physiological processes such as stomatal conductance and nutrient transport. The gradual decline in RWC from 30 to 120 mM NaCl, characterized by

overlapping statistical groupings, suggests that the plant maintains partial osmotic adjustment through compatible solute accumulation. However, at 150 mM NaCl, RWC dropped sharply and formed a statistically isolated group, indicating that compensatory mechanisms were overwhelmed and cellular dehydration became severe. Similar reductions in RWC under salinity stress have been associated with decreased hydraulic conductivity and altered aquaporin activity [18]. Zhu *et al*. [19] also suggested that reduced RWC may represent an adaptive response for maintaining osmotic balance under stress conditions. Since water status directly affects biomass production and secondary metabolism in medicinal plants, maintaining adequate RWC is essential for sustainable anise production under saline conditions.

The decline in chlorophyll a and b contents under salinity stress demonstrates the negative impact of salt stress on the photosynthetic apparatus of *P. anisum*. Salinity disrupts chlorophyll biosynthesis through ionic toxicity and oxidative stress caused by excessive Na^+ and Cl^- accumulation [6]. In addition, salinity may interfere with magnesium uptake and destabilize chloroplast membranes through lipid peroxidation [20]. Chlorophyll b exhibited a pronounced initial decline at 30 mM NaCl, after which it stabilized at lower levels across higher salinity treatments, whereas chlorophyll a showed a more continuous, dose-dependent reduction. This pattern suggests that light-harvesting complexes are particularly sensitive to early salt exposure, while overall pigment degradation progresses steadily as stress intensifies. Similar findings have been reported by Acosta-Motos *et al*. [20], who demonstrated that salinity affects both chlorophyll biosynthesis and degradation pathways. Reduced chlorophyll concentration ultimately limits photosynthetic efficiency, biomass accumulation, and secondary metabolite production.

Carotenoids play important roles in light harvesting and protection against oxidative damage. The observed decrease in carotenoid content under salinity stress indicates a progressive disruption of carotenoid biosynthesis coupled with increased utilization of these pigments for scavenging reactive oxygen species (ROS). Under saline conditions, enhanced ROS generation accelerates oxidative damage to chloroplast membranes and pigments [21]. The stepwise reduction in carotenoid means across salinity levels reflects a cumulative metabolic burden rather than a sudden collapse, indicating that *P. anisum* gradually depletes its antioxidant pigment reserves as salinity intensifies. At severe salinity, chloroplast damage and lipid peroxidation further reduce pigment stability. Similar reductions in carotenoid content under salinity stress have been reported in medicinal plants by Ahmed *et al*. [22] and Li *et al*. [21]. Therefore, carotenoid depletion may serve as a reliable indicator of salt sensitivity in anise plants.

The increase in soluble carbohydrate content under salinity stress indicates an important adaptive response in *P. anisum*. Soluble sugars contribute to osmotic adjustment, maintenance of cellular hydration, membrane stability, and protection against oxidative stress. The statistically distinct upward trend in carbohydrate means from control to 150 mM NaCl suggests continuous activation of stress-defense mechanisms, with the highest accumulation occurring under severe osmotic conditions. Similar responses were reported by Zhang *et al*. [23], while Gupta and Huang [24] suggested that soluble sugars also participate in stress signaling pathways. Therefore, carbohydrate accumulation appears to be a crucial biochemical strategy contributing to salinity tolerance in anise.

Proline accumulation is another common adaptive response to salinity stress. In the present study, proline content exhibited a biphasic pattern: a notable decrease at 30 mM NaCl relative to the control, followed by a significant and sustained increase from 60 mM NaCl onward, where all higher treatments formed a statistically homogeneous high-accumulation group. This indicates that initial mild stress may temporarily suppress proline metabolism, but moderate-to-severe salinity rapidly triggers robust biosynthetic pathways for osmotic and oxidative defense. Proline plays important roles in osmotic regulation, ROS detoxification, and membrane stabilization under stress conditions. Similar findings were reported by Kaur and Asthir [25] and Hasanuzzaman et al. [26], who associated proline accumulation with enhanced biosynthesis and reduced degradation under salt stress. The observed threshold-dependent increase in proline concentration confirms the activation of protective metabolic pathways in response to salinity stress in *P. anisum*.

The significant reduction in plant dry weight under increasing salinity levels reflects the inhibitory effects of salt stress on plant growth and metabolism. While dry weight at 30 mM NaCl did not differ significantly from the control, indicating initial growth resilience, progressive salinity led to statistically distinct biomass declines, culminating in the lowest mean at 90 mM NaCl. Severe salinity levels (120 and 150 mM) resulted in complete plant mortality, underscoring the relatively low salt tolerance threshold of *P. anisum*. Reduced biomass under salinity stress is generally associated with osmotic stress, ionic toxicity, impaired photosynthesis, and metabolic imbalance. However, the relative stability at lower salinity levels suggests that mild stress may still permit activation of protective mechanisms such as osmolyte accumulation and antioxidant defense. Previous studies have shown that moderate salinity may enhance secondary metabolite production despite reductions in biomass [23, 7].

Changes in essential oil content under salinity stress indicate considerable metabolic flexibility in *P. anisum*. Moderate salinity levels (30 and 60 mM NaCl) significantly increased essential oil content in both seeds and leaves, with the highest statistically distinct peaks occurring at 60 mM. This suggests that mild-to-moderate osmotic stress stimulates secondary metabolite biosynthesis, likely as a protective response. Similar observations were reported by Selmar and Kleinwächter [27]. However, at 90 mM NaCl, seed essential oil content dropped sharply to a statistically isolated minimum, while leaf content declined to an intermediate level. This divergence indicates that severe salinity triggers ionic toxicity, membrane damage, and disruption of terpenoid biosynthesis pathways, with reproductive tissues (seeds) being more severely affected than vegetative tissues (leaves). Similar biphasic responses have been reported by Farooq et al. [28] and Selmar and Kleinwächter [27]. These findings suggest that moderate salinity may enhance essential oil production in anise, whereas severe salinity negatively affects both growth and metabolic activity.

CONCLUSIONS

This study evaluated the physiological and biochemical responses of *P. anisum* to varying salinity levels. Increasing NaCl concentrations progressively reduced photosynthetic pigments and shoot dry weight, while relative water content remained stable until a sharp decline at 150

mM. Notably, plants subjected to ≥120 mM NaCl failed to complete their growth cycle, underscoring the species' limited tolerance to severe salinity. In contrast, moderate salinity (up to 60 mM) stimulated the accumulation of osmoprotectants (proline and soluble carbohydrates) and significantly enhanced essential oil production in both seeds and leaves, indicating a stress-induced metabolic adaptation. However, essential oil biosynthesis was markedly suppressed at 90 mM, particularly in seeds, highlighting a narrow optimal window for secondary metabolite enhancement. Given the high economic value of anise essential oil, these findings demonstrate that carefully managed mild-to-moderate salinity can be leveraged to improve phytochemical yield without compromising plant viability. Future research should focus on elucidating the molecular regulation of stress-responsive metabolite pathways and developing salt-resilient *P. anisum* cultivars through targeted breeding or agronomic optimization.

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Authors' contributions
E.N wrote the manuscript with support from Kh.A, the statistical analyst. S.A measured the physiological traits in the laboratory. All authors read and approved the final manuscript.

Conflict of Interest
Authors do not have any conflict of interest to declare.

Ethical Issues
None.

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