Improving drought tolerance and essential oil in *Nigella sativa* through salicylic acid seed priming

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Abstract

This research investigated how salicylic acid (SA) seed priming influences the growth performance and essential oil content of *Nigella sativa* L. when exposed to varying degrees of drought stress during the 2023–2024 season in Mahabad, Iran. A factorial experiment arranged in a randomized complete block design with three replications was implemented. Treatments involved three irrigation frequencies (7, 14, and 21 days) and four SA concentrations (0, 1000, 2000, and 3000 µM). The findings indicated significant impacts of both drought levels and SA applications on parameters such as plant height, capsule count per plant, seed number per capsule, biological and grain yield, and essential oil yield. Interaction between SA concentration and irrigation frequency significantly affected seed yield. Drought reduced all measured traits, whereas seed priming with 2000 µM SA consistently led to superior values. The combination of 14-day irrigation and 2000 µM SA was optimal for both seed and oil production. These outcomes highlight SA priming as a promising strategy for enhancing drought resilience in *Nigella sativa*.

Keywords: drought, essential oil, *Nigella sativa*, salicylic acid, seed priming

Nabizadeh, E., A. Esmzadeh, Kh. Ahmadi. 2025. 'Improving drought tolerance and essential oil in *Nigella sativa* through salicylic acid seed priming'. *Iranian Journal of Plant Physiology 1*5 (3),5657- 5666.

Introduction

Medicinal plants, particularly those with diverse biological activities, have garnered significant interest in both scientific and medical communities. Among them, *Nigella sativa* L., commonly known as black seed or black cumin and a member of the Ranunculaceae family, stands out for its extensive culinary and therapeutic applications (Kiralan et al., 2016). The widespread medicinal use of black seed, due to its efficacy in treating a variety of ailments, has attracted global attention - an interest partly rooted in historical accounts (Islam et al., 2019). In traditional medicine, roasted black seeds have long been used to treat conditions such as melancholia, fatigue, and persistent headaches. When mixed with honey or butter, these seeds have been recommended for relieving colic and cough. Their galactagogue and antiseptic properties have also made them effective in treating eye infections.

Seed germination represents a critical physiological function, serving as a prerequisite for the successful cultivation of most crops (Ghiyasi et al., 2019). Environmental stresses may adversely affect plant quality and yield potential,

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Received: May, 2025
Accepted: June, 2025

with water scarcity exerting a particularly detrimental influence on growth and metabolic processes (Alaghemand et al. 2019). In medicinal and aromatic plants, drought stress may significantly alter both yield and the composition of secondary metabolites (Tan and Gören, 2024, Fallah et al. 2019). Drought conditions lead to the accumulation of phenolic and flavonoid compounds, enhancing antioxidant capacity and lipid peroxidation in such plants (Nouraei et al., 2018).

Several commercially available chemical agents are used as elicitors to induce the synthesis of specific secondary metabolites in plants, thereby modulating their biological activity (Ramirez-Estrada et al., 2016). Among them, salicylic acid (SA) is a crucial phytohormone that regulates numerous developmental processes, including seed germination, pigment accumulation, stomatal movement, photosynthesis, thermogenesis, ethylene biosynthesis, enzymatic activity, nutrient uptake, membrane functionality, flowering induction, and metabolic activities (Monteiro et al., 2022). Due to its hormone-like properties, SA is widely employed to improve plant tolerance to both biotic and abiotic stresses and to stimulate the biosynthesis of secondary metabolites. This naturally occurring compound is found in plants at very low concentrations (Ali et al., 2021) and is thought to activate the phenylpropanoid pathway, resulting in increased synthesis of specific secondary compounds (Ninkuu et al., 2023). Studies have demonstrated that exogenous application of SA improves the accumulation of polyphenols and essential oils in Thymus vulgaris under water stress (Khalil et al., 2018). Additionally, Noori et al. (2022) reported that SA-treated cumin (*Cuminum cyminum*) exhibited improved nitrogen and sulfur utilization through increased nitrate reductase and ATP-sulfurylase activities and glutathione synthesis.

Considering the high medicinal value of black seed, its potential to adapt to adverse environmental conditions, and the need for efficient water resource management in agriculture, optimizing production under water-limited conditions is essential. Furthermore, limited data are available regarding seed priming effects under drought stress in *Nigella sativa*. Therefore, the present study was conducted to investigate the influence of seed priming using salicylic acid on selected morphological and physiological traits of *Nigella sativa* under drought conditions.

Materials and Methods

Experimental site and time

This study was conducted during 2023-2024 growing seasons at a research farm located in Mahabad, West Azerbaijan Province, Iran (latitude 36°57′ N, longitude 45°25′ E, altitude 1327 m above sea level). The region's climatic data for the 2023–2024 agricultural year indicates an average monthly temperature of 15.1 °C and an average monthly precipitation of approximately 102 mm (Table 1). The soil type at the site was loamy-sandy. Recorded summer temperatures ranged from a minimum of 14.5 °C to a maximum of 40.6 °C, and over the past two decades, average annual precipitation in the area ranged between 450-500 mm. Certified seeds with high viability were sourced from Pakan Seed Company (Isfahan, Iran), due to the suboptimal quality of seeds available in the local market. The salicylic acid used in this experiment (chemical formula $C_7H_6O_3$, 99% purity) was procured from Maharashtra, India.

Experimental design

The experiment was conducted using a factorial arrangement based on a randomized complete block design (RCBD) with three replications. The factors included irrigation intervals at three levels (7 days – control, 14 days – moderate stress, and 21 days – severe stress) and seed priming with salicylic acid at four concentrations (0, 1000, 2000, and 3000 µM).

Experimental implementation

The sowing was carried out in early June 2023. Each experimental plot consisted of eight rows, each 4 meters in length, with 30 cm spacing between rows and 7 cm spacing between plants on the row. A spacing of 1.5 meters was maintained between main plots and 3 meters between blocks to prevent moisture interference between adjacent plots. The soil texture at the site

Table 1
Average monthly temperature and precipitation during the agricultural year of the experiment (Mahabad City, 2023–2024)

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Precipitation (cm)	0	22.2	16.2	33.9	11.76	9.48	3.41	3.78	22	0	0	0
Temperature (°C)	15.8	12.7	3.7	9.3	3.5	9.3	12.9	14.4	21	27.5	27.7	23.6

was silty clay loam. Fertilization was based on soil test results, including 150 kg/ha of urea applied in two split doses (before sowing and at the stem elongation stage), 150 kg/ha of triple superphosphate, and 250 kg/ha of elemental sulfur, all incorporated into the soil prior to sowing using a rotavator.

Seed priming was conducted by foliar spraying with salicylic acid solutions at concentrations of 0, 1000, 2000, and 3000 µM. Irrigation treatments were applied at 7, 14, and 21-day intervals. To ensure precision in irrigation management, Parshall flumes were used to measure water discharge. These devices calculate flow rates based on water height measured at a defined location within the flume's converging throat and downstream expanding section. Their accuracy under standard irrigation conditions is approximately ±5%. Parshall flumes with throat widths ranging from 7.5 cm to 2.5 meters are suitable for flow rates from 0.8 liters per second to 4 cubic meters per second. They are ideal for monitoring water inflow into the plots and within furrows, as their design prevents sediment deposition, thus preserving measurement accuracy. Weed control was performed manually throughout the growing season. Salicylic acid treatments were applied via foliar spraying one week before the onset of drought stress (after full establishment of seedlings) and again three times at one-day intervals, starting three weeks after drought treatment initiation. Control plants were sprayed with distilled water. To improve adherence and absorption of the salicylic acid solution, 20% Tween was added as a surfactant.

Measured traits

At physiological maturity, several plants were randomly selected from each plot to measure plant height using a ruler. For determining the number of capsules per plant, seeds per capsule, and total yield, plants from each plot were harvested, bagged, and weighed using a digital scale. These measurements were expressed in grams per plot. To calculate capsule number, seed count per capsule, and 1000-seed weight, ten plants per plot were sampled.

Biological yield measurement

To determine biological yield, all harvested biomass from each plot was oven-dried at 70 °C until a constant weight was achieved, and then weighed.

Essential oil extraction

Essential oil was extracted using hydro-distillation via a Clevenger-type apparatus. For this purpose, 30 grams of crushed black cumin seeds were mixed with 300 mL of distilled water (10:1 ratio) in a round-bottom flask, and distilled for 3 hours at boiling temperature (Clevenger, 1928). Essential oil yield per unit area was calculated using the following formula:

Essential Oil Yield (kg/ha) = (Essential oil percentage × Seed yield in kg/ha) / 100

Statistical Analysis

The obtained data was submitted to SAS software (version 9.0, SAS Inc.) for analysis using Duncan's test at p≤0.05. The graphs were plotted using Excel.

Results

Plant height

The effects of drought stress and salicylic acid application on plant height were statistically significant while their interaction was not significant (Table 2). Comparison of treatment means revealed that the greatest plant height of *Nigella sativa* was observed under the 7-day

Table 2
Analysis of variance (ANOVA) for the effects of drought stress and salicylic acid on the studied traits of black cumin (*Nigella sativa*)

S.O.V	df	Mean square (MS)					
		Plant height	Capsules plant⁻¹	Seeds capsule⁻¹	Biological yield	Grain yield	Essential-oil yield
Replication	2	0.87	0.65	1.43	1269.87	1093.08	0.40
Irrigation interval (I)	2	994.65**	325.33**	1009.64**	547840.44**	165986.99**	48.16**
Salicylic acid (S)	3	193.58**	39.52**	103.77**	84261.93**	23022.40**	6.93**
I × S	6	5.61^{ns}	0.86^{ns}	0.23^{ns}	1787.77^{ns}	1821.87*	1.49^{ns}
Error	22	4.40	0.46	0.88	1978.06	691.67	1.03
C.V (%)	–	5.51	3.83	2.39	3.05	4.45	14.71

ns: not significant; *: significant at p≤0.05; **: significant at p≤0.01.

irrigation intervals, with an average height of 46.27 cm, whereas the lowest height, 28.27 cm, occurred under the 21-day intervals (Fig. I. a). Among the salicylic acid concentrations, the 2000 µM treatment produced the tallest plants, outperforming other concentrations. The average plant heights under 0, 1000, 2000, and 3000 µM salicylic acid were 33.79, 38.43, 44.40, and 35.63 cm, respectively (Fig. I. b). These results suggest that the 7-day irrigation interval is optimal for maximizing plant height in black cumin, and that seed priming with 2000 µM salicylic acid elicits a more beneficial physiological response in comparison to other concentrations. Drought stress significantly reduced plant height, probably due to inhibited cell expansion and reduced availability of essential nutrients under limited water conditions.

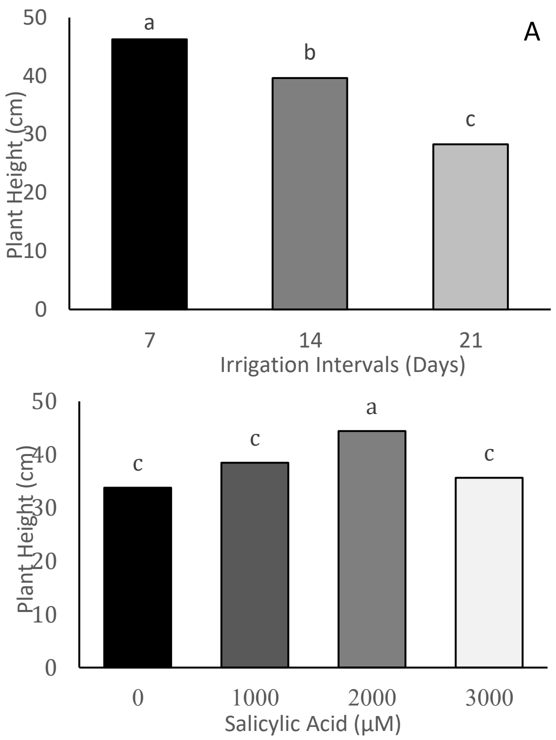

Fig. I. Comparison of the mean; a) effect of irrigation intervals; b) effect of salicylic acid on plant height of black cumin

Number of capsules per plant

According to the analysis of variance results, the effects of drought stress and salicylic acid on the number of capsules per plant were significant (Table 2). The mean comparison indicated that among the irrigation intervals, the 7-day interval resulted in a higher number of capsules per plant in

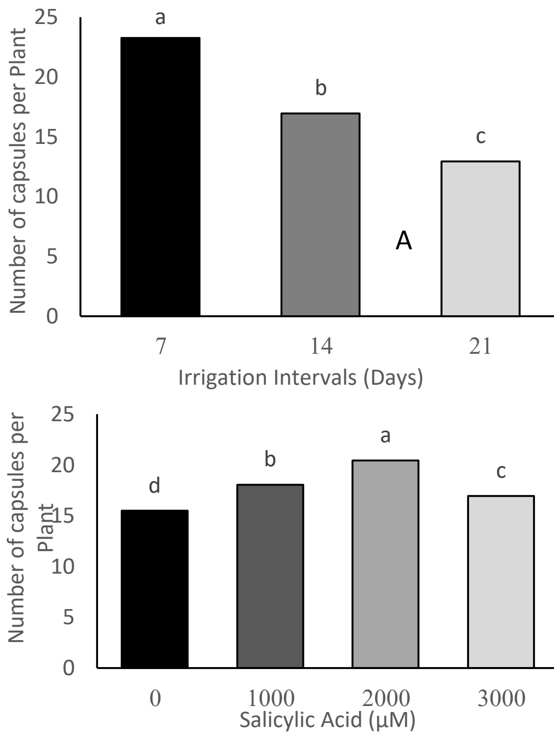

Fig. II. Comparison of the mean: a) effect of irrigation intervals; b) effect of salicylic acid on the number of capsules per plant in black cumin

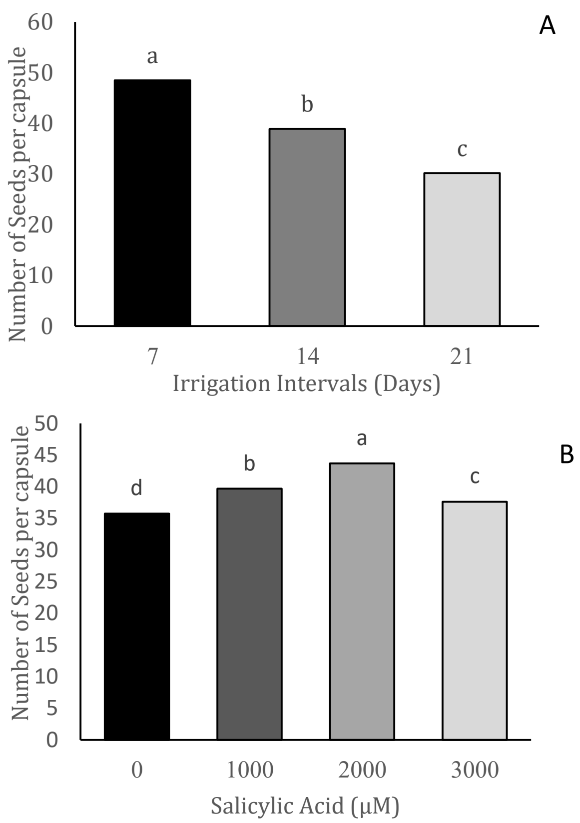

Fig. III. Comparison of the means: a) effect of irrigation intervals; b) effect of salicylic acid on the number of seeds per capsule in black cumin.

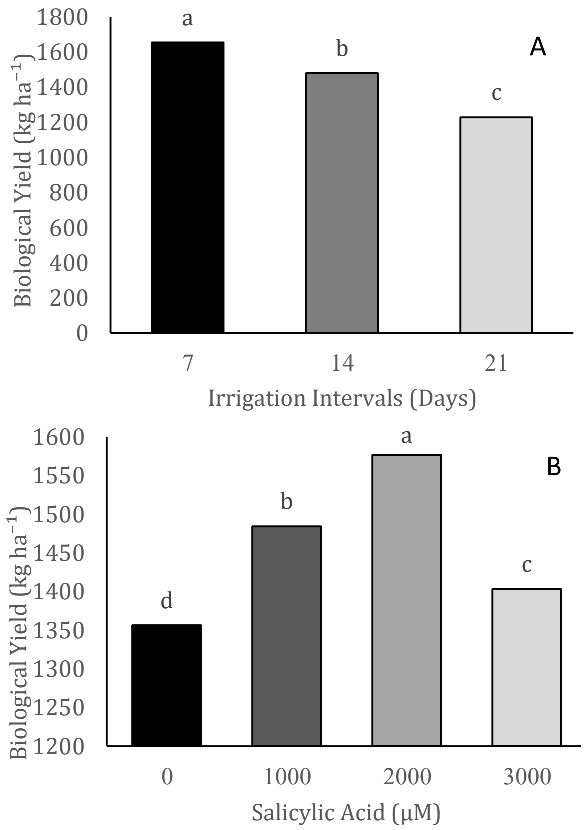

Fig. IV. Comparison of the means: a) effect of irrigation intervals; b) effect of salicylic acid on the biological yield of black cumin.

comparison to other intervals, with significant differences being observed among treatments (Fig. II. a). Additionally, among different concentrations of salicylic acid, the 2000 μM concentration led to the highest number of capsules per plant in comparison to the other concentrations (0, 1000, and 3000 μM), with values of 15.48, 18.05, 20.44, and 16.93 capsules respectively (Fig. II. b). Drought stress reduced the number of capsules per plant, probably due to water deficiency hindering the growth and development of reproductive structures. However, application of salicylic acid mitigated the negative effects of drought stress, leading to an increase in the number of capsules per plant.

Number of seeds per capsule

Analysis of variance indicated that the number of seeds per capsule was significantly affected by irrigation intervals and salicylic acid concentrations at 1% probability level (Table 2). Mean comparisons showed that the 7-day irrigation interval resulted in the highest number of seeds per capsule, with an average of 48.51 seeds while the 21-day interval had the lowest, with an average of 30.17 seeds, showing significant differences among treatments (Fig. III. a). Among salicylic acid concentrations, the 2000 μM concentration resulted in the highest number of seeds per capsule while the control (0 μM) had the lowest, with all four concentrations showing significant differences in this trait. Drought stress negatively affected seed yield components, leading to a decrease in final seed yield (Fig. III. b). Salicylic acid application improved yield and yield components of black cumin under 12-day irrigation intervals.

Biological yield

Based on the analysis of variance, biological yield was significantly affected by irrigation intervals and salicylic acid concentrations at 1% probability level (Table 2). Comparison of the means indicated significant differences among irrigation intervals, with the 7-day and the 21-day intervals resulting

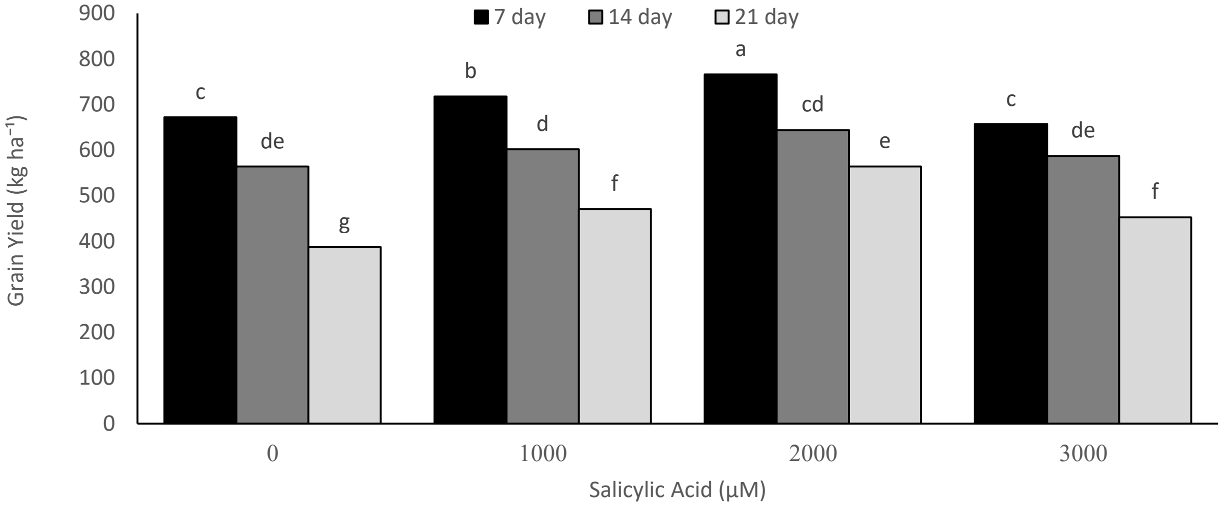

Fig. V. Comparison of the mean effect of irrigation and salicylic acid treatments on grain yield of black cumin.

in the highest (1654.99 kg/ha) and lowest (1229.99 kg/ha) biological yields, respectively, showing significant differences among treatments (Fig. IV. a). Among salicylic acid concentrations, the 2000 µM concentration resulted in the highest biological yield (1576.85 kg/ha) while the control (0 µM) had the lowest (1356.49 kg/ha), showing significant differences (Fig. IV. b).

Grain yield

According to the analysis of variance, grain yield was significantly affected by irrigation intervals, salicylic acid concentrations, and their interaction (Table 2). Comparison of the means showed that the highest grain yield was observed in the 7-day irrigation interval with the 2000 µM salicylic acid concentration, indicating significant differences among treatments (Fig. V). The 2000 µM salicylic acid concentration was the most effective across all irrigation intervals while the control (0 µM) resulted in the lowest grain yield.

Essential oil yield

Essential oil yield was significantly affected by irrigation intervals and salicylic acid concentrations at the 1% probability level (Table 2). Comparison of the means showed significant differences among irrigation intervals, with the 7-day interval resulting in the highest essential oil yield (8.87 kg/ha) and the 21-day interval the lowest (4.87 kg/ha) (Fig. VI. a). Among salicylic acid concentrations, the 2000 µM concentration

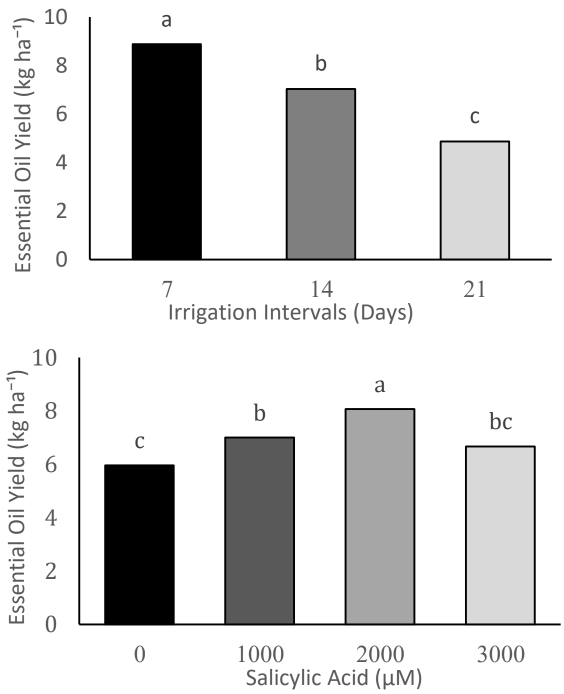

Fig. VI. Comparison of the means: a) effect of irrigation intervals; b) effect of salicylic acid on essential oil yield of black cumin

resulted in the highest essential oil yield (8.07 kg/ha) while the control (0 µM) had the lowest (5.96 kg/ha). No significant difference was observed between the 0 and 3000 µM concentrations but both differed Significantly from the other treatments (Fig. VI. b).

Discussion

The results of this study demonstrate that both drought stress and salicylic acid (SA) application significantly influenced the growth, yield, and essential oil production of *Nigella sativa*, although their interaction was only significant for certain traits, such as grain yield. These findings underscore the physiological importance of SA under abiotic stress, particularly water deficit. Plant height notably reduced under drought conditions, particularly at the 21-day irrigation interval, likely due to impaired cell expansion and reduced nutrient uptake caused by limited water availability. Conversely, SA application, especially at 2000 µM, significantly increased plant height. This improvement can be attributed to SA's role as a signaling molecule that modulates physiological processes including cell division, elongation, and metabolism (Li et al., 2022). At low to moderate concentrations, SA has been shown to stimulate vegetative growth in various crops, such as *Capsicum annuum* and *Brosimum alicastrum*, under both normal and stress conditions (Tucuch-Haas et al., 2017).

Drought stress led to a significant decrease in the number of capsules per plant and seeds per capsule, likely due to reduced energy availability for reproductive development. However, SA application at 2000 µM mitigated these adverse effects, promoting higher capsule and seed numbers. This is consistent with the well-documented role of salicylic acid (SA) in enhancing photosynthetic capacity and promoting assimilate partitioning toward reproductive organs under stress conditions (Fan et al., 2022; Awadalla et al., 2024). Improved reproductive traits following SA treatment have also been reported in various drought-stressed crops, including pea, maize, and wheat (Xin et al., 2023). Biological yield decreased substantially under extended irrigation intervals, highlighting the negative impact of prolonged water scarcity on overall biomass production.

As irrigation intervals increased, grain priming with salicylic acid at appropriate concentrations could mitigate the detrimental effects of drought stress, whereas unprimed grains suffered significant yield losses, highlighting the importance of salicylic acid priming. High concentrations of salicylic acid may damage plants while low concentrations improve antioxidant production and strengthen the plant's defense system against free radicals induced by stress, thereby increasing yield.

The highest biological yield was observed under the 7-day irrigation interval and 2000 µM SA treatment. This may be due to enhanced root growth, water uptake, and photosynthetic efficiency facilitated by SA (Islam et al., 2019). Moreover, SA is known to stabilize cellular membranes and promote the accumulation of lignin and other structural components, contributing to higher biomass under stress conditions (Xin et al., 2023). The application of salicylic acid probably contributed to the expansion and health of the root system, improved water and nutrient uptake, and improved photosynthesis in leaves, thereby positively influencing the biological yield of black cumin. Additionally, the increase in biological yield due to salicylic acid application may be attributed to its antioxidant activity in cell membranes, leading to increased lignin content in cell wall structures, which may contribute to increased biomass weight, especially under drought stress conditions. Drought stress reduced biological yield due to water deficiency hindering biomass production.

Grain yield was significantly affected by both drought stress and SA application, with a notable interaction between the two. The combination of frequent irrigation and 2000 µM SA produced the highest yield while the absence of SA under water-deficient conditions resulted in the lowest. This suggests that SA not only improves plant resilience but also enhances productivity by maintaining photosynthetic activity and facilitating efficient translocation of assimilates to the grains. Similar outcomes have been reported in safflower (*Carthamus tinctorius*), where foliar application of salicylic acid under drought or saline conditions significantly increased seed yield, oil content, and harvest index by mitigating oxidative damage and enhancing antioxidant enzyme activities (e.g., CAT, POX, SOD) (Naghizadeh and Maghsoudi, 2024). The negative effects of reduced irrigation on seed yield components in this study could be attributed to decreased vegetative growth, limited photosynthetic area, and reduced dry matter production, ultimately leading to lower final seed yield under drought conditions. On the other hand, the exogenous application of salicylic acid probably prevented the reduction in seed number and weight by enhancing photosynthesis in leaves and subsequent storage of photosynthates in seeds. A similar beneficial effect was observed in Allium species as in shallot (*Allium hirtifolium*), foliar SA application under water-deficit conditions improved leaf relative water content, chlorophyll retention, membrane stability, and bulb yield by over 15% compared to untreated drought control (Yousefvand et al., 2022). Essential oil yield also decreased under severe drought, with the 21-day irrigation interval resulting in the lowest production. This decline is possibly due to the diversion of photosynthates toward osmoprotectants like proline and sugars rather than secondary metabolite synthesis (Ellouzi et al., 2023). However, the 2000 µM SA treatment significantly improved essential oil yield, likely by enhancing plant metabolic activity and maintaining biosynthetic pathways for secondary metabolites even under stress.

This study demonstrated that drought stress and seed priming with salicylic acid significantly affected key physiological and agronomic traits of *Nigella sativa*. Both factors individually influenced plant height, capsule number, seed number per capsule, biological yield, seed yield, and essential oil content while their interaction significantly impacted seed yield. The combination of 7-day irrigation intervals and 2000 µM salicylic acid consistently resulted in superior plant performance across all measured parameters. This treatment notably improved biomass accumulation, reproductive output, and essential oil yield in comparison to other salicylic acid concentrations and irrigation regimes. Overall, seed priming with 2000 µM salicylic acid appears to be an effective strategy for improving drought resilience and productivity in *Nigella sativa* under water-limited conditions.

Acknowledgements

The authors would like to express their sincere gratitude to the Research and Technology Deputy of Islamic Azad University, Mahabad Branch, for providing the facilities and support required to carry out this experiment. We also thank the Department of Agriculture, Faculty of Agriculture in Mahabad for their assistance with field operations and data collection.

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